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# *Fungal Contamination in Public Buildings:*

# *Health Effects and Investigation Methods*

Canada

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# *Fungal Contamination in Public Buildings:*

# *Health Effects and Investigation Methods*

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## Abstract

The word "mold" is a non-scientific term that in popular parlance generally refers to members of a few dozen filamentous fungi. Mold growth on building surfaces not only damages these surfaces, but also affects air quality as intact spores, as well as spore and mycelial fragments, are dispersed in the air. These can be inhaled depending on their size and concentration. Exposure to mold is associated with increased rates of respiratory disease.

This document is a revision of an earlier version published by Health Canada and the Federal-Provincial Advisory Committee on Environmental and Occupational Health (CEOH) in 1995. The intent is to update the information and to reconcile certain practical aspects of the document with newer publications from the American Conference of Governmental Industrial Hygienists (ACGIH), the American Industrial Hygiene Association (AIHA) and other cognizant authorities. The purpose of this document is to assist front-line public health workers in the management of potential health risks associated with fungal contamination in public buildings. The report consists of two parts:

1. A review on health effects of indoor molds
2. A guide for the investigation of mold contamination in non-industrial workplaces

### 1. Health Effects of Indoor Molds

The 1995 review concluded that ". . . epidemiological studies have consistently detected an association with respiratory symptoms and home dampness and mold growth, but causality in these studies has not been established." The purpose of this section is to update the CEOH document by reviewing the research published from 1995 to 2001 on health effects of exposure to molds in residences and non-industrial workplaces (mostly office buildings and schools), and to determine whether the current evidence warrants more definitive conclusions.

Major findings from this review are:

- Eight cross-sectional studies investigated the relationship between indoor mold and respiratory, allergic or irritation symptoms, four of which found significant association between mold exposure and either physician-diagnosed asthma or asthma-related symptoms (cough, wheezing or breathlessness).
- Seven case-control studies investigated the relationship between mold and asthma, most relying only on self-reports to assess both mold exposure and health outcomes. One of these studies found a significant association between "mold or dampness" and asthma; another found a significant association between mold and asthma but did not assess dampness; three found significant associations between mold and asthma (one of them after controlling for dampness) but not between dampness and asthma; and two found significant associations between dampness and asthma, but not between mold and asthma.
- To date, no cohort studies have been published on the association between residential mold exposure and asthma, although a published study has found an association between mold exposure at school and childhood asthma. There is presently an ongoing cohort study in Prince Edward Island, Canada.
- Several experimental studies with animal models exposed to fungal cells, antigens or constituents have found effects similar to those observed in humans in epidemiological studies, such as eosinophilia and increased serum IgE.

Several of the studies reviewed were limited by the methods used: exposure and outcome assessment based on self-reporting; no quantitative exposure assessment (and therefore no determination of a dose–response relationship); possible confounding by other biological agents; and potential response bias.

Only in a few studies reported to date has an independent effect of mold on asthma and upper respiratory health been demonstrated. Therefore, from epidemiologic data alone, it is difficult to assess the population health consequences of the material growth of indoor molds. It is known, however, that exposure to fungi in occupational environments causes allergic and toxic diseases. Adverse effects of fungi have also been seen in inhalation studies using animal models. Therefore, further investigation of health effects of indoor fungi using improved exposure and health outcome assessment methods is needed to resolve uncertainties. As established by the CEOH in 1995, current knowledge indicates the need to prevent damp conditions and mold growth and to remediate any fungal contamination in buildings.

### 2. Investigation of Fungal Contamination in the Non-Industrial Workplace

It cannot be emphasized enough that the best way to manage mold growth is to prevent it before it occurs. The essential elements of a prevention strategy are control of moisture, timely remediation of any water leakage, and adequate maintenance of heating, ventilation and air conditioning (HVAC) systems.

The goals of a mold investigation are to:

- establish the cause, nature and extent of fungal contamination;
- assess the risk of adverse effects on the health of building occupants;
- manage the microbial problem(s); and
- return the building to a satisfactory level of performance.

The first step in investigating a building for microbial contaminants is an informed inspection. Mold contamination can arise from condensation, floods and various types of leaks. Investigation of mold problems requires a thorough knowledge of the design of the building envelope and the types of failures that result in condensation and water leaks. Where there is probable cause to believe that there is appreciable mold behind wall cavities, physical inspections should be performed by opening up the hidden area.

Air sampling is appropriate either during or following the inspection. The main purpose of such sampling is to identify contamination that would not be visible without destructive testing and to document air contamination. Air samples should be taken during normal activity in the building, while the ventilation system is operational. They should be collected simultaneously inside and outside the building to enable indoor–outdoor comparisons. The basis of the current methods for interpreting the results of air sampling is a comparison of the diversity of the fungi inside with outdoor air samples.

Sticky surface samplers are increasingly used in mold investigations. Advantages of data from properly collected and analyzed sticky surface samples are twofold: the results are available within a day and in situations when there is a high percentage of non-viable spores in the air, the data are more reliable.

Once the investigation is completed, fungal damage should be expeditiously remediated using state-of-the-art protocols such as those developed by the New York City Department of Health and the ACGIH. As well, quality assurance should be carried out according to standard protocols such as those of the AIHA.

Communication with buildings managers and occupants should be maintained throughout the investigation.

## Preamble

In 1993, the Federal-Provincial Advisory Committee on Environmental and Occupational Health (CEOH) released *Indoor air quality in office buildings: a technical guide.* This report provided guidance on methods for investigating buildings for air quality problems, including molds. A subsequent report from the same committee, *Fungal Contamination in Public Buildings: A Guide to Recognition and Management* (1995), provided information on health implications of molds in buildings and a step-by-step protocol for the investigation and interpretation of indoor fungal contamination.

The purpose of this current document is to update the *Fungal Contamination in Public Buildings* report in view of the large amount of research reported since 1995 on health effects of mold damage in the built environment, as well as on methods for investigating buildings for such damage. This report is not, therefore, intended to replace the *Technical Guide*, but to provide additional information to those responsible for the investigation and management of fungal contamination in office buildings, schools and other non-industrial workplaces.

Consistent with the 1995 report, this updated review of health effects indicates that living or working in a building with material mold damage is harmful to health. Therefore, indoor mold growth in buildings should be prevented by appropriate control of moisture sources and by timely remediation of water damages. Mold growing in buildings should also be removed under safe conditions using established remediation protocols.

A significant difference between the two documents is the greater emphasis on the general principles of investigation in the current report. As new building investigation techniques become validated, the general principles described here can be used as a framework for their application.

# 1. Introduction

Photo: Canada Mortgage and Housing Corporation (CMHC)

## 1.1 Indoor Air as a Public Health Issue

The potential impacts of indoor air contamination on human health have received considerable public attention in recent years. This is especially true in Canada, and other countries with cold weather, where people spend most of their time indoors. Indoor air can be contaminated by pollutants released from carpets and building materials, cleaning chemicals, tobacco smoke, cooking and heating, as well as biological contaminants such as dust mite and animal allergens (derived from skin, saliva and urine) and molds. This report discusses only one aspect of this complex array of contaminants: mold. Much of what is known about the population health effects of biological contaminants in indoor environments comes from studies of people living in damp homes.

Living in damp houses is associated with increased rates of disease, and the cause is believed to be exposure to biological contaminants (Institute of Medicine 2000). Occupants in houses that have dampness problems are at greater risk of exposure to mold, dust mites and bacterial endotoxins. Lower socio-economic status has been associated with higher prevalence of respiratory disease (Dales *et al.* 2002). In most countries, poverty translates into living in substandard housing that leaks water and air, and is difficult to heat. When houses are difficult or expensive to heat or cool, the air in some rooms is often not conditioned. This leads to moisture accumulation (condensation) on cold surfaces. Depending on the surfaces and degree of house cleaning, contaminants accumulate and airborne particulate concentrations will vary accordingly. Due to complex exposures, however, the attributable risk to each of the biological contaminants discussed here remains unknown. This alone makes it difficult to assign tolerable exposure values.

In addition, outdoor-source fine particles ($PM_{2.5}$) can be higher indoors than outdoors. Houses near sources of outdoor air pollution (e.g. vehicular traffic) are at greater risk of increased indoor concentrations of particulate matter and volatile organic compounds (VOCs).

Among indoor air contaminants, mold is a cause of increasing concern, with many epidemiological studies and case reports linking mold to a wide range of adverse effects on respiratory health.

## 1.2 What Is Mold?

The fungus kingdom consists of eukaryotic organisms. Fungi are subdivided into four different phyla based on their reproduction mode: ascomycetes, basidiomycetes, zygomycetes and mitosporic fungi.

The word "mold" is a non-scientific term that in popular parlance generally refers to members of a few dozen filamentous fungi. Such fungi are often visible as colonies on food and building materials, appearing on close inspection as multicellular filaments called *hyphae*. Mold growth on building material surfaces can influence air quality because both spores and mycelial fragments are dispersed into the air and can be inhaled, depending on their size.

The spores of fungi have a large size range: 1 to 50 $\mu$m. Furthermore, the degree of hydration of spores, a consequence of the prevailing relative humidity, affects this range (Madelin and Johnson 1992). Particles at the lower end of the size range (less than 10 $\mu$m) can reach the alveoli; others may be swallowed. There is some variation with age: lower airway deposition for 5 $\mu$m particles is six times higher in newborns than in adults (Phalen and Oldham 2001). The average aerodynamic diameter of a number of spore types is listed in Table 1. The average sizes of some spores are well within the respirable range (<10 $\mu$m); others such as *Stachybotrys chartarum* appear to be too large to penetrate into the lungs. However, there is considerable variation not represented by the average. For example, even though the average aerodynamic diameter of *Stachybotrys* spores is too large to penetrate into the lungs, approximately one third of the spores are within the respirable range (Sorenson *et al.* 1996). Similar data for some strains of *Cladosporium cladosporioides*, *Penicillium viridicatum* and *P. chrysogenum* showed a large range in spore sizes whereas most spores of *P. commune*, *Aspergillus versicolor*, *A. ustus*, *A. niger* and *A. sydowii* were of similar dimensions (Miller and Young 1997). As noted, mycelial fragments are also typically present in indoor air. These are usually of respirable size. The number of fragments compared to the number of spores present is highly variable, but typically represent a few percent of the fungal particles present. It is known that mycelial fragments of some species contain different allergens than those present in spores of the same species (Górny *et al.* 2002).

Table 1.
Spore size of various fungi determined by cascade impaction and microscopy

| Species | Average aerodynamic diameter µm | Axial dimensions µm |
|---|---|---|
| *Aspergillus fumigatus* | 2.2 | 2.2×2.5 |
| *Cladosporium cladosporioides* | 2.2 | 1.8–3.6 (1.1–2.2 × 2.2–4.6) |
| *Paecilomyces lilacinus* | 2.7 | 2.4–3.1 × 2.4–3.1 |
| *Penicillium chrysogenum* | 3.0 | 2.8–4.2 × 2.8–4.2 |
| *Memnoniella echinata* | 4.8 | 4.8–6.2 × 4.8–6.2 |
| *Stachybotrys chartarum* | 4.8 | 4.8–6.2 × 4.8–6.2 |

(After Madelin and Johnson 1992; Somsen *et al.* 1996)

Table 2
Common fungi from mold-damaged building materials

| | |
|---|---|
| *Aspergillus versicolor* | *Memnoniella echinata* |
| *Aspergillus fumigatus* | *Paecilomyces variotii* |
| *Aspergillus ochraceus* | *Penicillium brevicompactum* |
| *Chaetomium globosum* | *Penicillium chrysogenum* |
| *Cladosporium cladosporioides* | *Penicillium commune* |
| *Cladosporium sphaerospermum* | *Penicillium corylophilum* |
| *Eurotium amstelodami* | *Scopulariopsis brevicaulis* |
| *Eurotium repens* | *Stachybotrys chartarum* |

(Adapted from Flannigan *et al.* 2001)

Three features of mold biochemistry are of special interest in terms of human health. First, mold cell wall contains (1->3)-ß-D-glucan, a compound with inflammatory properties. Second, spores and mycelial fragments contain allergens (Górny *et al.* 2002), few of which have been chemically characterized. Many of the known fungal allergens are serine proteases, or proteins, which are present in fairly high concentrations in the spores. These have been described mainly from work done in phylloplane species and *Aspergillus fumigatus* (Horner *et al.* 1995). Third, the spores of some species contain low molecular weight chemicals that are cytotoxic or have other toxic properties (e.g. satratoxins produced by *Stachybotrys chartarum*). Some molds, such as *Aspergillus fumigatus*, can cause opportunistic infection in immunocompromised individuals and severe allergic diseases in people with underlying respiratory conditions, such as asthma or cystic fibrosis (Burge 2000). Fungi commonly found in moldy building materials are shown in Table 2.

*Aspergillus versicolor*

*Penicillium chrysogenum*

*Stachybotrys chartarum*

Photos: Centraalbureau voor Schimmelcultures, Koninklijke Nederlandse Academie van Wetenschappen, The Netherlands

# 2. Health Effects of Indoor Molds

Photo: Dr. Amanda Wheeler

Since 1982, in Europe and North America, approximately 30 studies have been conducted on the association between dampness, mold and respiratory health in residential housing. Studies in the United States and Canada have involved the largest number of people. A study of the respiratory health of 4,600 children from six cities in the northeast United States demonstrated that the presence of mold and dampness in their homes was correlated to several respiratory symptoms as well as a number of non-specific symptoms. The effect on the children was of similar dimension to parental smoking (Brunekreef *et al.* 1989). Two studies involving 15,000 children and 18,000 adults from 30 communities in Canada came to similar conclusions. The authors suggested that a non-allergenic mechanism may be involved since there was no effect modification by reported atopy and asthma. A dose–effect relationship was also seen in that more visible mold yielded more symptoms. Overall, the mold contamination was associated with a 50% relative increase in asthma and a 60% increase in upper respiratory disease (Dales *et al.* 1991a, 1991b). Data from a further 13,000 children from 24 cities across the United States (19 cities) and Canada (5 cities) show the same pattern (Spengler *et al.* 1994). The upper boundary attributable risk for mold-caused asthma in Canada was estimated at 20% (Dekker *et al.* 1991). The health effects of fungal contamination in housing remain significant even after adjustment for socio-economic factors, pets, household smokers, endotoxins and dust mites (Dales and Miller 1999; Dales *et al.* 1999).

A review published in 1995 by Health Canada and the Federal-Provincial Advisory Committee on Environmental and Occupational Health (CEOH) concluded that ". . . epidemiological studies have consistently detected an association with respiratory symptoms and home dampness and mold growth, but causality in these studies has not been established" (CEOH 1995a). The evidence linking exposure to indoor molds with adverse respiratory outcomes has also been reviewed by Verhoeff and Burge (1997). More recently, the US National Academy of Sciences Institute of Medicine released a report on asthma entitled *Clearing the Air: Asthma and Indoor Air Exposures.* The panel found that there was insufficient evidence on a population health basis for the association between indoor residential molds and the development of asthma, but that indoor mold was associated with exacerbation of asthma in mold-sensitized individuals, and exposure may be associated with respiratory symptoms. The percentage of mold-sensitized asthmatics is not known; estimates range up to 40% (Institute of Medicine 2000).

The purpose of this section is to update the review conducted by the Federal-Provincial Advisory Committee on Environmental and Occupational Health (CEOH) in 1995 by reviewing the research published since then on health effects due to exposure to molds in residences and non-industrial workplaces (mostly office buildings and schools), and to determine whether the current evidence warrants more definitive conclusions. Following a summary of studies published since 1995 (section 2.1), some potential effects of molds in sensitive sub-populations are discussed (section 2.2), followed by an overview of the experimental studies on respiratory effects of molds (section 2.3) and a discussion of the evidence linking mold exposure to adverse health outcomes (section 2.4).

Health problems, such as hypersensitivity pneumonitis (HP) and organic dust toxic syndrome (ODTS) identified in industrial and agricultural settings due to greater exposure to molds (and, in some instances, other biological contaminants such as thermophilic actinomycetes), will not be discussed here.

## 2.1 Epidemiological Studies of Respiratory Illness

In order to review recent cross-sectional and cohort studies on health effects of indoor molds, Medline was searched using the following keywords: fungi, or mold, or mold and respiratory tract diseases. Articles published in 1995 or later pertaining to cross-sectional, cohort or case-control studies assessing the association between indoor exposure to molds (visible mold growth or airborne fungal cell counts) and asthma or related respiratory symptoms were included in the review. Studies with no mold exposure variable (e.g. those considering only dampness) and prevalence studies with no measure of association were excluded.

### 2.1.1 Cross-sectional studies

Cross-sectional studies are studies in which outcomes (diseases) and exposures are assessed at one point of time. Eight cross-sectional studies, summarized in Table 3, investigated the relationship between indoor mold and respiratory, allergic or irritation symptoms, and four found significant association between mold exposure and either physician-diagnosed asthma or asthma-related symptoms (cough, wheezing or breathlessness).

Table 3.
Cross-sectional studies on respiratory and allergic effects of exposure to indoor molds, 1995 to 2001

| Country | Study population (n) | Data collection method | Exposure |
|---|---|---|---|
| Canada | School children, mean age = 10 (n = 403) | E + D: questionnaire to parents | Mold/mildew in present home in the past year |
| Canada | As above | E: dust sampling<br>D: questionnaire to parents | *Alternaria* detected in dust |
| Australia | Children between 7 and 14 years of age from 80 households (n = 148) | E: air sampling<br>D: questionnaire (asthma) and skin prick test to common aeroallergens (atopy) | 100-CFU/m³ increase in *Penicillium* spores |
| | | | 100-CFU/m³ increase in *Aspergillus* spores |
| Finland | Adults aged 25 to 64 years (n = 1,460) | E + D: mail questionnaire | Visible mold OR musty odour OR moisture stains OR water/moisture damage |
| Finland | As above, but excluding those reporting lumbar backache or recurrent stomachache | E + D: mail questionnaire | Visible mold OR musty odour OR moisture stains OR water/moisture damage |

E: exposure assessment; D: disease assessment

| Disease | OR | 95% CI | Covariats adjusted for | Ref. |
|---|---|---|---|---|
| Non-respiratory symptoms | 2.25 | 1.26 - 4.00 | Age, gender, parental allergies, parental education, pets, ETS, dust mites, bacterial endotoxins | Dales and Miller 1999 |
| Irritation | 1.81 | 1.02 - 3.24 | | |
| Cough/wheezing | 1.28 | 0.74 - 2.23 | | |
| Asthma | 0.91 | 0.47 - 1.95 | | |
| Chest illness | 1.51 | 0.76 - 3.02 | | |
| Non-respiratory symptoms | 0.79 | 0.38 - 1.67 | Age, parental illness, parental smoking, dust mites | Dales *et al.* 1999 |
| Irritation | 1.05 | 0.51 - 2.18 | | |
| Cough/wheezing | 2.00 | 0.84 - 4.74 | | |
| Asthma | 1.90 | 0.55 - 6.59 | | |
| Chest illness | 2.77 | 0.85 - 9.01 | | |
| Asthma | 1.43 | 1.03 - 2.00 | Parental asthma, allergy | Garrett *et al.* 1998 |
| Atopy (response to at least one skin prick test) | 1.48 | 1.10 - 1.99 | Gender, parental asthma | |
| Asthma | 1.02 | NS | Age, gender, smoking, education, type of dwelling | Pirhonen *et al.* 1996 |
| Atopy | 1.62 | p = 0.001 | | |
| Allergic rhinitis | 1.66 | p = 0.001 | | |
| Cough | 1.37 | NS | | |
| Phlegm | 1.36 | p < 0.05 | | |
| Rhinitis | 1.69 | p < 0.001 | | |
| Eye irritation | 1.52 | p = 0.01 | | |
| Lumbar backache | 1.49 | p < 0.01 | | |
| Recurrent stomachache | 1.65 | p = 0.001 | | |
| Atopy | 1.34 | NS | | Pirhonen *et al.* 1996 |
| Allergic rhinitis | 1.37 | NS | | |
| Cough | 1.48 | NS | | |
| Phlegm | 0.94 | NS | | |
| Rhinitis | 1.21 | NS | | |
| Eye irritation | 1.89 | p < 0.05 | | |

Table 3.
Cross-sectional studies on respiratory and allergic effects of exposure to indoor molds, 1995 to 2001 (continued)

| Country | Study population (n) | Data collection method | Exposure |
|---|---|---|---|
| Finland | Adults aged >= 16 years inhabiting 310 dwellings (n = 699) | E + D: self-administered questionnaire | Mold present |
| Finland | Adults aged >= 16 years inhabiting 310 dwellings (n = 699) | E: homes visually inspected for signs of moisture by a civil engineer<br>D: self-administered questionnaire | Moisture present |
| Finland | First-year university students aged 18 to 25 (n = 10,677) | E + D: postal questionnaire (D validated against clinical assessment in a sub-sample of 290 people) | Visible mold growth in dwellings in the past year |
| | | | Visible mold OR damp stains OR water damage in the past year |
| Netherlands | Boys aged 6 to 12 (n = 222) | E + D: questionnaire to parents (mold growth: never, sometimes, often, always) | Mold growth in past 2 years (always vs. never) |

| Disease | OR | 95% CI | Covariats adjusted for | Ref. |
|---|---|---|---|---|
| Sinusitis | 1.92 | 1.11 - 3.30 | Smoking, age, gender, allergy, pets, atopic predisposition | Koskinen *et al.* 1999 |
| Bronchitis | 1.98 | 1.13 - 3.48 | | |
| Cough without phlegm | 1.40 | 0.82 - 2.19 | | |
| Cough with phlegm | 1.15 | 0.78 - 1.69 | | |
| Nocturnal cough | 2.11 | 1.21 - 4.98 | | |
| Nocturnal dyspnoea | 2.33 | 1.09 - 4.98 | | |
| Sore throat | 1.46 | 1.03 - 2.08 | | |
| Rhinitis | 1.06 | 0.71 - 1.59 | | |
| Impaired smell | 1.23 | 0.80 - 1.91 | | |
| Eye irritation | 1.08 | 0.76 - 1.53 | | |
| Bronchitis | 1.68 | 0.95 - 2.95 | As above | Koskinen *et al.* 1999 |
| Cough without phlegm | 1.60 | 1.01 - 2.53 | | |
| Cough with phlegm | 1.44 | 1.44 - 2.19 | | |
| Nocturnal cough | 2.30 | 1.32 - 4.01 | | |
| Nocturnal dyspnoea | 1.58 | 0.74 - 3.39 | | |
| Sore throat | 2.40 | 1.56 - 3.69 | | |
| Rhinitis | 1.89 | 1.15 - 3.11 | | |
| Impaired smell | 1.28 | 0.80 - 2.06 | | |
| Eye irritation | 1.43 | 0.84 - 1.83 | | |
| Asthma | 2.21 | 1.48 - 3.28 | Parental education, smoking, ETS exposure, pets, wall-to-wall carpets, place of residence (farm, rural non-farm, urban), type of residence | Kilpeläinen *et al.* 2001 |
| Allergic rhinitis | 1.29 | 1.01 - 1.66 | | |
| Allergic conjunctivitis | 0.95 | 0.68 - 1.34 | | |
| Atopic dermatitis | 1.31 | 0.96 - 1.79 | | |
| Asthma | 1.66 | 1.25 - 2.19 | | |
| Allergic rhinitis | 1.30 | 1.12 - 1.51 | | |
| Allergic conjunctivitis | 1.12 | 0.92 - 1.36 | | |
| Atopic dermatitis | 1.29 | 1.06 - 1.56 | | |
| Chronic cough | 3.56 | 0.60 - 14.10 | Age, gender, parental smoking, unvented kitchen geysers, parental education | Culpers *et al.* 1995 |
| Shortness of breath | 2.26 | 0.54 - 9.49 | | |
| Wheezing | 0.95 | 0.16 - 5.46 | | |

Table 3
Cross-sectional studies on respiratory and allergic effects of exposure to indoor molds, 1995 to 2001 (continued)

| Country | Study population (n) | Data collection method | Exposure |
|---|---|---|---|
| Netherlands (continued) | Girls aged 6 to 12<br>(n = 248) | E + D: questionnaire to parents (mold growth: never, sometimes, often, or always) | Mold growth in past 2 years (always vs. never) |
| Taiwan | Children aged 6 to 12<br>(n = 1,340) | E + D: questionnaire to parents | Dampness |
| Taiwan | Children aged 6 to 12<br>(n = 1,340) | E + D: questionnaire to parents | Mold growth in home |
| United States | Young adults aged 20 to 22 years<br>(n = 2,041) | E + D: self-administered postal questionnaire | Visible mold |
| | | | Water leaking |
| | | | Indoor dampness |

| Disease | OR | 95% CI | Covariats adjusted for | Ref. |
|---|---|---|---|---|
| Chronic cough | 0.79 | 0.07 - 8.34 | | |
| Wheezing | 2.69 | 0.48 - 15.21 | | |
| Cough | 2.52 | 1.34 - 4.75 | Age, gender, parental education, number of smokers in household, gas stove | Li and Hsu 1996 |
| Phlegm | 1.86 | 1.18 - 2.93 | | |
| Wheeze | 1.36 | 0.83 - 2.21 | | |
| MD-diagnosed asthma | 1.25 | 0.81 - 1.95 | | |
| Bronchitis | 1.29 | 0.96 - 1.73 | | |
| Pneumonia | 1.33 | 0.75 - 2.36 | | |
| Allergic rhinitis | 1.39 | 1.05 - 1.84 | | |
| Cough | 1.67 | 1.00 - 3.25 | As above | Li and Hsu 1996 |
| Phlegm | 1.50 | 0.95 - 2.36 | | |
| Wheeze | 1.20 | 0.73 - 1.99 | | |
| MD-diagnosed asthma | 1.12 | 0.72 - 1.74 | | |
| Bronchitis | 1.68 | 1.26 - 2.25 | | |
| Pneumonia | 1.77 | 1.03 - 3.05 | | |
| Allergic rhinitis | 1.27 | 0.96 - 1.68 | | |
| MD-diagnosed asthma | 1.5 | 1.0 - 2.4 | Gender, race, education, smoking status | Hu *et al.* 1997 |
| Current asthma | 2.0 | 1.2 - 3.2 | | |
| MD-diagnosed asthma | 1.6 | 0.7 - 3.5 | | |
| Current asthma | 1.6 | 0.7 - 3.8 | | |
| MD-diagnosed asthma | 1.2 | 0.8 - 1.9 | | |
| Current asthma | 1.3 | 0.7 - 2.2 | | |

- In the Netherlands, 470 children aged 6 to 12 were included in a survey of home environment and respiratory symptoms. Mold growth in homes in the previous two years was classified as per its frequency: "never," "sometimes," "often" or "always." Odds ratios were adjusted for age, gender, parental smoking, unvented kitchen geysers and parental education. The study found that neither chronic cough, shortness of breath nor wheezing were associated with mold growth (Cuijpers *et al.* 1995).
- In Finland, 1,460 people aged 25 to 64 were included in a survey of home environment and respiratory symptoms. Data were collected through a mail-out questionnaire. Odds ratios were adjusted for age, gender, smoking, education and dwelling type. Living or having lived in a home with a dampness or mold problem, defined as visible mold, mold smell, moisture stains or water damage, was associated with bronchitis (OR 2.04, 95% CI 1.49 to 2.78), atopy (OR 1.63, 95% CI 1.26 to 2.10), allergic rhinitis (OR 1.66, 95% CI 1.25 to 2.19), phlegm (OR 1.36, 95% CI 1.01 to 1.85), rhinitis (OR 1.69, 95% CI 1.31 to 2.18) and eye irritation (OR 1.52, 95% CI 1.18 to 1.96), but not physician-diagnosed asthma (OR 1.02, 95% CI 0.60 to 1.72). Some non-respiratory, non-allergic diseases also showed significant associations with dampness or molds: fatigue (OR 1.81, 95% CI 1.37 to 2.39), lumbar backache (OR 1.49, 95% CI 1.15 to 1.93) and recurrent stomachache (OR 1.65, 95% CI 1.24 to 2.20). When data were re-analyzed after excluding "complainers," defined as those who reported lumbar backache and/or recurrent stomachache, in order to control for reporting bias, only ORs of eye irritation and fatigue remained significant (Pirhonen *et al.* 1996).
- In Taiwan, 1,340 children aged 8 to 12 years were surveyed. Data were collected through a questionnaire to parents. Odds ratios were adjusted for age, gender, parental education, number of smokers in household, and gas stove. Mold growth in homes was significantly associated with cough (OR 1.87, 95% CI 1.00 to 3.25), bronchitis (OR 1.68, 95% CI 1.26 to 2.25) and pneumonia (OR 1.77, 95% CI 1.03 to 3.05), but not physician-diagnosed asthma (OR 1.12, 95% CI 0.72 to 1.74) (Li and Hsu 1996).
- In the United States, 2,041 people aged 20 to 22 years who had responded to a mail-out questionnaire were included in a cross-sectional study. Data collection was based entirely on the questionnaire. Odds ratios were adjusted for gender, race, education and smoking status. Visible mold growth at home was associated with an increased risk of physician-diagnosed asthma (OR 1.5, 95% CI 1.0 to 2.4) and current asthma (OR 2.0, 95% CI 1.2 to 3.2) (Hu *et al.* 1997).
- In Australia, 80 households with 148 children aged 7 to 14 were surveyed. A detailed housing characterization was carried out, and air samples were collected. Also, a respiratory health questionnaire was completed and skin prick tests with extracts of common aeroallergens were performed for each of the children. The odds ratio for reported physician-diagnosed asthma with a 100-CFU/m$^3$ increase in *Penicillium* spores was 1.43 (95% CI 1.03 to 2.00), and the odds ratio for atopy, defined as a positive response to at least one skin prick test, with a 10-CFU/m$^3$ increase in *Aspergillus* spores was 1.48 (95% CI 1.10 to 1.99) (Garrett *et al.* 1998).
- In Finland, 699 adults aged at least 16 years were included in a cross-sectional study. Data collection consisted of a self-administered questionnaire followed by an investigation by civil engineers of mold and dampness in participants' dwellings. Odds ratios were adjusted for smoking, age, gender, allergy, indoor pets and atopy. The presence of mold in homes reported by occupants was associated with increased risk of cough without phlegm (OR 1.60, 95% CI 1.01 to 2.53), nocturnal cough (OR 2.30, 95% CI 1.32 to 4.01), sore throat (OR 2.40, 95% CI 1.56 to 3.69) and rhinitis (OR 1.89, 95% CI 1.15 to 3.11). The presence of molds observed by the civil engineers visiting the house was associated with an increased risk of sinusitis (OR 1.92, 95% CI 1.11 to 3.30), bronchitis (OR 1.98, 95% CI 1.13 to 3.48), nocturnal cough (OR 2.11, 95% CI 1.21 to 4.98), nocturnal dyspnea (OR 2.33, 95% CI 1.09 to 4.98) and sore throat (OR 1.46, 95% CI 1.03 to 2.08) (Koskinen *et al.* 1999).
- In Canada, the homes of 403 school children were surveyed. Parents filled out a questionnaire about their home environment and the respiratory health of their children. Air samples were collected and analyzed for ergosterol, viable fungi and bacterial endotoxin, while dust samples were collected for dust mite antigen analysis. Odds ratios were adjusted for age, gender, parental allergies and asthma, parental education, pets in homes and household smokers. Mold or mildew growth in the home in the past year was associated with irritation of eyes, nose or skin (OR 1.80, 95% CI 1.03 to 3.16), but not cough or wheezing (OR 1.36, 95% CI 0.79 to 2.33) or physician-diagnosed asthma (OR 0.96, 95% CI 0.46 to 2.00). Additional adjustment for bacterial endotoxin and dust mites did not change the magnitude of these associations. No significant association was found between ergosterol and fungal cell counts, and respiratory outcomes (Dales and Miller 1999; Dales *et al.* 1999).

- In Finland, 10,677 first-year university students aged 18 to 25 were included in a questionnaire-based survey. Exposure and outcome assessments were based on responses. Odds ratios were adjusted for parental education, active and passive smoking, pets, carpets, place of residence (farm, rural non-farm or urban) and type of residence. Visible mold in participants' dwelling in the past year was associated with current physician-diagnosed asthma (OR 2.21, 95% CI 1.48 to 3.28), common cold at least four times in the past year (OR 1.48, 95% CI 1.17 to 1.88) and allergic rhinitis (OR 1.29, 95% CI 1.01 to 1.66) (Kilpeläinen *et al.* 2001).

### 2.1.2 Case-control studies

In case-control studies, exposure is assessed and compared between subjects with the disease of interest (cases), and without this disease (controls). Nine case-control studies, summarized in Table 4, have investigated the relationship between mold and asthma, most of them relying only on self-reports to assess both mold exposure and health outcomes. One of these studies found a significant association between "mold or dampness" and asthma; another found a significant association between mold and asthma, but did not assess dampness; three found significant associations between mold and asthma (one of them after controlling for dampness), but not between dampness and asthma, and two found significant associations between dampness and asthma, but not between mold and asthma. Interestingly, these two studies used objective criteria rather than self-reports to assess health outcomes, and home inspection for assessing exposure or validating the exposure questionnaire.

- In the Netherlands, a nested case-control study was carried out within a random sample of 7,632 children aged 6 to 12 years whose parents had completed a screening questionnaire. Cases were selected among children with reported asthma (n=76), chronic cough (n=81) or other respiratory conditions, for a total of 259. Controls (n=257) were selected among children without respiratory symptoms. Data were collected through a self-administered questionnaire to parents and through a visit to all participants' homes by a trained investigator blind to children's case or control status. Crude odds ratios were calculated: reported mold somewhere in the houses was associated with an increased risk of chronic cough (OR 1.90, 95% CI 1.02 to 3.52), reported mold in the living room with physician-diagnosed asthma (OR 2.95, 95% CI 1.34 to 6.52) and reported mold in the bedroom with chronic cough (OR 3.52, 95% CI 1.55 to 8.03). No significant association was found with the presence of mold observed by the investigator. Adjusted odds ratios were also calculated, and were mostly lower than the corresponding crude odds ratios. When cases with elevated Imunoglobulin E (IgE) antibodies to molds and/or dust mites were compared to controls without IgE to these allergens, mold exposure observed by the investigator was found to be associated with sensitization + physician-diagnosed asthma (crude OR 2.61, 95% CI 1.21 to 5.64) and sensitization + chronic cough (crude OR 3.45, 95% CI 1.20 to 9.93) (Verhoeff *et al.* 1995).
- In the United Kingdom, 486 cases who had frequent or speech-limiting wheezing in the previous 12 months, and 475 controls, selected from participants in a previous health survey, were included in a case-control study. Participants were aged 11 to 16 years. Exposure classification was based on damp or mold in the children's bedroom: "none," "damp only" and "damp with mold." In univariate analysis, no association was found between damp bedroom (without mold) and asthma (unadjusted OR 0.85, 95% CI 0.39 to 1.83), but the presence of both dampness and mold in the bedroom was associated with an increased risk of wheezing (crude OR 2.20, 95% CI 1.11 to 4.43). For the multivariate analysis, the mold/dampness variable was dichotomized into "none" and "any mold," and was no longer associated with wheezing (Strachan and Carey 1995).
- In the United Kingdom, 102 patients with physician-diagnosed asthma, aged 5 to 44 years and 196 population controls were interviewed by a trained interviewer about their respiratory health and their housing conditions. After the interview, subjects were asked to have their house inspected by a surveyor blind of their case or control status, and 222 out of 298 participants agreed. Odds ratios were adjusted for age, gender, income, unemployment, smoking, other smokers living in homes, and pets. When self-reported exposure was considered (283 participants included), asthma was associated with the presence of "any dampness" (OR 1.93, 95% CI 1.14 to 3.28) or "severe dampness" (OR 5.45, 95% CI 2.81 to 10.6), with dampness in previous home (OR 2.55, 95% CI 1.49 to 4.37), and with having moved because of dampness in previous home (OR 2.08, 95% CI 1.02 to 4.24). When exposure observed by the surveyor was considered, asthma was associated with the presence of "any dampness" (OR 3.03, 95% CI 1.65 to 5.57) and "severe dampness" (OR 2.36, 95% CI 1.34 to 4.01), but not with the presence of mold (OR for "severe mold": 1.70, 95% CI 0.78 to 3.71) (Williamson et al. 1997).

Table 4.
Case-control studies on asthma and exposure to indoor molds, 1995 to 2001

| Country | Study population | Cases/controls definition | Data collection method |
|---|---|---|---|
| Canada | Children aged 5 to 19 years whose parents responded to a previous survey | **Cases (n = 592)**: physician-diagnosed asthma AND risk of the following: wheeze in past year, use of asthma medication<br>**Controls (n = 443)**: no history of asthma | D + E: report by parents of a self-made checklist |
| Austria | Children whose parents responded to the ISAAC phase two survey | **Cases (n = 1,782)**: wheezing in the past 12 months<br>**Controls (n = 29,966)**: no wheezing in past 12 months | E + D: questionnaire to parents |
| Finland | Adults aged 21 to 63 years | **Cases (n = 521)**: newly diagnosed asthma, adult<br>**Controls (n = 932)**: no previous or current asthma | E + D: questionnaire |
| Netherlands | Infants (median age 2.4 y) + 12 years, whose parents completed a respiratory symptoms questionnaire | **Cases (n = 78)**: physician-diagnosed asthma<br>**Controls (n = 257)**: no respiratory symptoms | E + D: questionnaire to parents<br>E: home inspection<br>D: questionnaire to parents |
| Netherlands | As above | **Cases**: physician-diagnosed asthma AND IgE sensitivity to mold and/or dust mites<br>**Controls**: no respiratory symptoms | E: questionnaire to parents<br>D: questionnaire to parents (asthma) and blood IgE (allergy)<br>E: home inspection<br>D: questionnaire to parents (asthma) and blood IgE (allergy) |

E: exposure assessment, D: disease assessment

| Exposure | OR | 95% CI | Covariates adjusted for | Ref |
|---|---|---|---|---|
| Mold and/or mildew in home in the past year | 1.6 | 1.1 - 2.3 | Gender, age, gas cooking, maternal asthma and allergies, number of siblings | Hesso et al 2001 |
| Dampness or mold at home | 1.43 | 1.24 - 1.65 | Age, gender, family history of asthma, parental education, ETS exposure | Zacharasiewicz et al 1999 |
| **Work** | | | Gender, age, parental atopy, education, smoking, ETS, occupational exposures, pets and all variables shown | Jaakkola et al. 1992 |
| Visible mold on job site | 1.64 | 1.07 - 2.52 | | |
| Damp storage/basement working | 1.84 | 1.09 - 3.10 | | |
| Water damage | 2.47 | 1.63 - 3.74 | | |
| **Home** | | | | |
| Visible mold/mildew | 2.60 | 1.68 - 4.01 | | |
| Damp storage/basement dwelling | 1.53 | 1.03 - 2.27 | | |
| Water damage | 2.30 | 1.49 - 3.54 | | |
| Dampness in house | 1.90 | 1.30 - 2.80 | No adjustment in the Office | Verhoeff et al. 1995 |
| Dampness in bedroom | 1.87 | 1.36 - 2.56 | | |
| Dampness in living room | 1.41 | 1.03 - 2.00 | | |
| Mold in house | 1.87 | 1.34 - 2.61 | | |
| Mold in living room | 2.16 | 1.54 - 3.03 | | |
| Mold in bedroom | 1.80 | 1.31 - 2.47 | | |
| Dampness in house | 1.87 | 1.37 - 2.55 | | |
| Dampness in bedroom | 1.84 | 1.37 - 2.48 | | |
| Dampness in living room | 1.33 | 1.00 - 1.77 | | |
| Mold in house | 1.80 | 1.35 - 2.39 | | |
| Mold in living room | 1.98 | 1.51 - 2.61 | | |
| Mold in bedroom | 1.69 | 1.31 - 2.19 | | |
| Dampness in house | 1.96 | 1.48 - 2.58 | No adjustment in the Office | Verhoeff et al. 1995 |
| Mold in house | 1.99 | 1.56 - 2.47 | | |
| Dampness in house | 1.82 | 1.49 - 2.21 | | |
| Mold in house | 2.91 | 2.31 - 3.64 | | |

Table 4.
Case-control studies on asthma and exposure to indoor molds, 1995 to 2001 (continued)

| Country | Study population | Cases/controls definition | Data collection method |
|---|---|---|---|
| Sweden | Individuals aged 20 to 44 who participated in a previous survey | **Cases (n = 98)**: wheezing or breathlessness in past year AND bronchial hyperresponsiveness to methacholine<br>**Controls (n = 357)**: no current asthma | E: questionnaire validated against inspection by an occupational hygienist in a sub-sample of 68 dwellings (mold: Cohen's kappa, = 0.36)<br>D: symptoms questionnaire and bronchial challenge test with methacholine |
| Sweden | Adults aged 20 to 50 years who responded to short respiratory survey **(n = 15,813)** | **Cases (n = 174)**: physician-diagnosed asthma after age 16<br>**Controls (n = 870)**: randomly selected regardless of their asthma status (including 35 fulfilling the case definition) | E + D: self-administered questionnaire |
| United Kingdom | Children aged 11 to 16 whose parents had responded to a previous survey | **Cases (n = 486)**: frequent or speech-limiting wheezing in the past 12 months<br>**Controls (n = 475)**: no history of asthma or wheezing | E + D: questionnaire to parents |
| | As above, but excluding those with "changes to bedroom as a result of asthma or allergy" | **Cases (n = 365) and Controls (n = 463)**: see definition above | As above |
| United Kingdom | | **Cases (n = 102)**: hospital patients with physician-diagnosed asthma, aged 5 to 44 years<br>**Controls (n = 196)**: population controls matched for sex and age within 5 years | E: questionnaire<br>D: hospital records (validity) |
| | | | E: home visits by a surveyor (222 subjects only)<br>D: hospital records (validity) |

| Exposure | OR | 95% CI | Covariates adjusted for | Ref. |
|---|---|---|---|---|
| Water damage or flooding | 1.8 | 1.0 - 3.2 | Age, gender, smoking | Norbäck *et al.* 1999 |
| Dampness in the floor | 4.6 | 2.0 - 10.5 | | |
| Visible mold on indoor surfaces | 1.9 | 0.93 - 3.8 | | |
| Moldy odour | 1.1 | 0.42 - 2.9 | | |
| At least one sign of building dampness | 1.8 | 1.1 - 3.0 | | |
| Visible dampness | 1.3 | 0.9 - 2.0 | Age, gender, smoking habits, atopy | Thorn *et al.* 2001 |
| Visible mold growth | 2.2 | 1.4 - 3.5 | | |
| Visible dampness and mold growth | 1.8 | 1.1 - 3.1 | | |
| Visible mold growth | 2.4 | 1.3 - 4.2 | As above + visible dampness | |
| Dampness only | 0.85 | 0.39 - 1.83 | None (crude ORs) | Strachan and Carey 1995 |
| Dampness with mold | 2.20 | 1.11 - 4.43 | | |
| Mold | 1.25 | 0.67 - 2.31 | Age, gender, type of pillow and quilt, age of mattress, gas cooking, parental smoking | |
| Any dampness | 1.93 | 1.14 - 3.28 | Age, gender, household income, unemployment, smoking, ETS exposure, pets | Williamson *et al.* 1997 |
| Serious dampness | 5.45 | 2.81 - 10.6 | | |
| Dampness in previous home | 2.55 | 1.49 - 4.37 | | |
| Moved because of dampness | 2.08 | 1.02 - 4.24 | | |
| Any dampness | 3.03 | 1.65 - 5.57 | | |
| Severe dampness | 2.36 | 1.34 - 4.01 | | |
| Any mold | 1.35 | 0.79 - 2.28 | | |
| Significant mold | 1.70 | 0.78 - 3.71 | | |

- In Sweden, a nested case-control study was carried out among individuals aged 20 to 44 who participated in a respiratory health survey, and who agreed to undergo a lung function examination and a bronchial challenge test with methacholine, and to provide a blood sample. Ninety-eight cases had current asthma, defined as a combination of bronchial hyperresponsiveness and either wheezing or breathlessness in the past 12 months, and 357 controls had no current asthma. Exposure assessment was based on questionnaire data. Odds ratios were adjusted for age, gender and smoking. Current asthma was associated with water damage or flooding in dwellings (OR 1.8, 95% CI 1.002 to 3.2) and dampness signs on the floor (OR 4.6, 95% CI 2.0 to 10.5), and a similar but non-significant trend was found for visible mold on indoor surfaces (OR 1.9, 95% CI 0.93 to 3.8). A stratified random sample of 88 dwellings was inspected by an occupational hygienist who recorded signs of building dampness as requested in the questionnaire. There was a good agreement between participants' and the occupational hygienist's report on water damage (Cohen's Kappa 0.40, $\rho$=0.001) and visible mold (Cohen's Kappa 0.36, $\rho$=0.004) (Norbäck *et al.* 1999).
- In Austria, a subset of 1,781 participants in a health survey of children aged 6 to 9 years was used for a case-control study. Cases were those who answered "yes" to the question "wheezing in the past 12 months?" and controls were those who answered "no" to that question. Odds ratios were adjusted for age, gender, parents' or sibling's history of asthma, parental education and exposure to environmental tobacco smoke. Dampness or mold at home was significantly associated with wheezing (OR 1.43, 95% CI 1.24 to 1.65) (Zacharasiewicz *et al.* 1999).
- In Sweden, a nested case-control study was carried out among respondents aged 20 to 50 years to a questionnaire-based survey. Cases were those who reported asthma diagnosed by a physician at age 16 or older, and controls were randomly selected among the survey participants. Selected participants were sent a comprehensive questionnaire regarding their health, their home environment and other risk factors. Odds ratios were computed using a logistic regression controlling for gender, sex, smoking habits and atopy. Visible mold growth in any of the six latest homes inhabited was significantly associated with asthma (OR 2.2, 95% CI 1.4 to 3.5), and this association remained significant after controlling for visible dampness (OR 2.4, 95% CI 1.3 to 4.2) (Thorn *et al.* 2001).
- In Canada, a nested case-control study was carried out among children aged 5 to 19 in two communities in Alberta. Participants were selected from among those whose parents had responded to a mail-out questionnaire. Five hundred and ninety-two cases were randomly selected among those with a current physician-diagnosed asthma, and 443 controls were selected among those with no history of asthma. Data on demographic, environment, medical history and host factors were collected by telephone interviews. Odds ratios and confidence intervals were calculated using unconditional logistic regression, including potential confounders: gender, age, gas cooking, parental asthma and allergies, and number of siblings. Exposure to indoor molds or mildew in the past year was significantly associated with asthma (OR 1.6, 95% CI 1.1 to 2.3) (Hessel *et al.* 2001).
- In Finland, a case-control study was carried out in adults aged 21 to 63 years. Cases were newly diagnosed asthma cases and controls had no previous or current asthma. Odds ratios were adjusted for gender, age, parental atopy or asthma, education, smoking, environmental tobacco smoke exposure, pets and occupational exposures. No association was found between dampness or mold exposure in the home and asthma. Conversely, visible mold or mold odour at work was associated with a higher risk of adult-onset asthma (OR 1.54, 95% CI 1.01 to 2.32). No such association was found between dampness or water damage, and asthma (Jaakkola *et al.* 2002).

### 2.1.3 Building investigations

Sick building syndrome (SBS) describes a series of symptoms with no clear etiology, such as eye, nose and throat irritation, headaches and high frequency of airway infection and cough, which are associated with a building environment. It is distinguished from building-related illnesses (BRI) which are well-defined responses to biological, physical or chemical exposures occurring in indoor environments (Brightman and Moss 2000). SBS and BRI investigations were mostly cross-sectional (i.e. comparing occupants of buildings where problems were identified to those of "control" buildings). Some of these studies included a longitudinal component, as the health of exposed individuals was reassessed after exposure had been eliminated.

Some of these studies where mold contamination was investigated, along with other exposures, are summarized here. It should be kept in mind that because of their cross-sectional design and some other methodological issues (multiple concomitant exposures, possible bias in studies

initiated following complaints), these studies cannot identify or demonstrate an independent association between an exposure such as mold and dampness, and a health outcome.

- In the United States, a health questionnaire was administered to 53 office workers with more than three months' employment in a water-damaged building where *Stachybotrys* growth was found, and to 21 office workers with similar duties, working in other buildings. Blood samples were also collected from every participant for immunologic tests. Employees from the water-damaged building had a significantly higher prevalence of lower respiratory problems (76% vs. 43%; $p<0.01$), and eye symptoms such as burning, irritation and blurry vision (57% vs. 19%; $p<0.01$). There was no difference between the two groups with respect to total white blood cell counts, but the proportion of eosinophils was marginally higher among employees from the water-damaged building ($p=0.06$) (Johanning *et al.* 1996).
- In the United States, a health questionnaire was administered to workers employed in three buildings with severe water damage and contamination with *Aspergillus versicolor* and *Stachybotrys chartarum*, and to workers from two control buildings with no visible mold growth. In total, 197 people participated in that study. Workers from the water-damaged buildings had a higher risk of eye itching and watering (OR 2.49, 95% CI 1.02 to 6.27), stuffy or blocked nose (OR 4.48, 95% CI 1.93 to 10.68), runny nose (OR 3.06, 95% CI 1.16 to 8.53), dry throat (OR 3.74, 95% CI 1.42 to 10.42), lethargy (OR 10.62, 95% CI 4.50 to 22.10), difficult breathing (OR 13.47, 95% CI 1.90 to 72.83), and chest tightness (OR 10.41, 95% CI 1.46 to 62.83) (Hodgson *et al.* 1998).
- In Finland, 397 children from a water-damaged, mold-contaminated school (thereafter referred to as "School E") were compared to 192 children from a control school where inspection revealed no mold contamination ("School C"). All participants were aged 7 to 12 years. Questionnaires were sent to parents of children from both schools before and after remediation in School E, and a physician reviewed diagnosis and antibiotic prescriptions in the children's medical records. Before remediation, children from School E had a higher risk of common cold (OR 1.51, 95% CI 1.04 to 2.20) and bronchitis (OR 2.76, 95% CI 1.11 to 6.81), but these differences disappeared after mold remediation in School E (Savilahti *et al.* 2000).

### 2.1.4 Cohort studies

In cohort studies, subjects classified according to their exposure are followed over time to determine the incidence of the disease of interest. To date, no cohort studies have been published on the association between residental mold exposure and asthma, although a published study has investigated the association between mold exposure at school and childhood asthma (see below). In addition, there is an ongoing cohort study in Prince Edward Island, Canada.

In Sweden, a prospective study was carried out over four years; a total of 1,347 children was surveyed twice, in 1993 and in 1997. Their mean age in 1993 was 10.3 years. Participants were attending 39 different schools at the time of the first survey. Total mold concentrations were determined in 1993 and 1995 and ranged from 5 to 360 cells/m$^3$ (arithmetic mean 26 cells/m$^3$). After adjustment for sex, age, atopy in 1993, and smoking, the odds ratios for incident asthma (i.e. diagnosed during the follow-up period) per 10-fold increase in total mold levels in classrooms was 1.3 (95% CI 0.5 to 3.6). Among children who were not atopic in 1993, the odds ratio for incident asthma per 10-fold increase in mold levels, adjusted for sex, age and smoking, was 4.7 (95% CI 1.2 to 18.4) (Smedje and Norbäck 2001).

## 2.2 Effects of Molds in Sensitive Groups

Some sub-populations have been found to be at increased risk of developing rare conditions following exposure to molds. Exposure to extremely high mold contamination has been associated with pulmonary hemorrhages in infants, and increased risk of invasive mycose has been observed in people with immune suppression.

### 2.2.1 Pulmonary hemorrhage

Exposure to indoor molds has been a suspected cause of idiopathic pulmonary hemorrhage in infants and young children. In most cases, the suspected etiologic agent was *Stachybotrys chartarum* (also known as *S. atra*), a hydrophilic fungus (i.e. requiring very damp conditions to grow) that produces cellulase and is therefore able to use cellulose as a substrate. *Stachybotrys chartarum* produces at least four families of compounds: atranones, macrocyclic trichothecenes, spirolactones and cyclosporin-like compounds (Sakamoto *et al.* 1993; Jarvis *et al.* 1995; Hinkley *et al.* 1999). There appear to be two chemotypes present in North American strains: those that produce all of the

families of compounds and those that do not produce tricho-thecenes, but do produce the others. Both types appear to occur together (Nielsen *et al.* 2002).

In Cleveland, Ohio, 10 infants aged less than one year were diagnosed with pulmonary hemorrhages and hemosiderosis between January 1993 and December 1994. Each of these cases was matched for age with three controls. Data collection was performed by a questionnaire administered to parents and sampling of molds on surfaces and in the air. Mean concentrations of viable mold conidia in the air were higher in houses of cases compared to houses of controls (total viable fungi: 29,227 CFU/m$^3$ vs. 707 CFU/m$^3$; *Stachybotrys chartarum*: 43 CFU/m$^3$ vs. 4 CFU/m$^3$). A 10-CFU/m$^3$ increase in the concentration of viable *Stachybotrys chartarum* conidia was associated with a significantly increased risk of acute pulmonary hemorrhage (OR 9.83, 95% CI 1.08 $3\times10^2$). Nine out of 10 cases lived with smokers, compared to 16 out of 30 controls (OR 7.9, 95% CI 0.9 to 70.6), suggesting that exposure to environmental tobacco smoke may act synergistically with the factors associated with damp buildings (Montaña *et al.* 1997; Etzel *et al.* 1998). A review panel mandated by the US Centers for Disease Control and Prevention (CDC) to reassess this investigation concluded that the methodology used to collect mold samples and to calculate airborne counts of viable spores was inappropriate (CDC 2000).

No other published epidemiologic study has investigated the association between exposure to *S. chartarum* and pulmonary hemorrhage, but cases of pulmonary hemorrhage have been reported in infants and young children exposed to it (Elidemir *et al.* 1999; Flappan *et al.* 1999) or to other hydrophilic, cellulolytic fungi (Novotny and Dixit 2000). In the Cleveland hospital where the initial outbreak occurred, 30 infants were hospitalized with acute pulmonary hemorrhage between 1993 and 2002. Twenty-six out of 29 infants lived in water-damaged buildings, and 25 out of 28 in homes containing toxigenic fungi (Dearborn *et al.* 2002).

In 2000, the CDC created three new working groups to develop better protocols for investigation of future clusters. Briefly, a review of patient records from the Cleveland cases by pediatric and other specialists indicated that there were no known potential causes for the disease reported in the original studies. A clear case definition was developed should any additional clusters of infant pulmonary hemorrhage be detected. Most of the babies included in the original studies and subsequent infants studied would be included by the new definition (Dearborn *et al.* 2002). A second working group concluded that the fungal exposure assessments in the original study were inadequate. Several investigation techniques were described in case of future reports of clusters of idiopathic pulmonary hemorrhage. Some of these techniques would not have been available at the time of the original investigation (CDC 2001). A third group developed a protocol for surveillance and CDC has begun a surveillance program in conjunction with the states (CDC 2004).

### 2.2.2 Invasive mycoses

Some fungi such as *Aspergillus* species are ubiquitous in the environment, and inhalation of their spores is very common. However, invasive mycoses (i.e. fungal infections) occur mostly in immunosuppressed patients. The most common pathogen is *Aspergillus fumigatus* (Bennett 1994). The incidence of invasive mycosis is increased in AIDS patients; an analysis of medical records of 35,232 HIV-infected patients who attended outpatients clinics in 10 US cities between 1990 and 1998 revealed that the incidence of invasive aspergillosis was 5.1 per 1000 (95% CI 2.8 to 7.3) in those with CD4 counts 50 to 99 cells/mm$^3$ and 10.2 per 1000 in those with CD4 counts lower than 50 cells/mm$^3$, compared to 1.0 per 1000 (95% CI 0.6 to 1.4) in those with CD4 counts equal to or higher than 200 cells/mm$^3$ (Holding *et al.* 2000).

Several outbreaks of invasive aspergillosis have been reported in hematology wards where neutropenic leukemia patients were housed. The risk of invasive aspergillosis in immunosuppressed patients was associated with the airborne concentrations of *Aspergillus* spores, and increased incidences have been observed following events resulting in higher *Aspergillus* counts in the air, such as construction or dysfunction in air filtration systems.

- From May 1981 to October 1985, 14 bone marrow transplant patients developed nosocomial *Aspergillus* infections out of 111 patients who underwent such transplants. Further analysis revealed that all these cases of infection occurred among the 74 patients housed outside a high-efficiency particulate air (HEPA) filtered environment, while none of the 39 patients housed in a HEPA-filtered environment developed aspergillosis. Only one of the 166 air samples collected in the HEPA-filtered environment (0.6%) was positive for *Aspergillus*, while 75 out of 466 samples collected elsewhere in the hospital (16.1%) and 13 out of 54 samples collected outside the hospital (24.1%) were positive (Sherertz *et al.* 1987).
- In 1993, six cases of aspergillosis were identified among the patients who attended the hematology-oncology ward of a pediatric hospital in Glasgow,

United Kingdom, while only one case had been identified in that hospital over the five previous years. The outbreak investigation revealed that a contaminated vacuum cleaner used in the ward dispersed a bioaerosol; the *Aspergillus* concentration close to that vacuum cleaner was 62 CFU/m$^3$ when it was in use, compared to 0 to 6 CFU/m$^3$ elsewhere in the building (Anderson *et al.* 1996).

- In the hematology-oncology ward of a Montréal hospital, the incidence of invasive aspergillosis in patients with leukemia or bone marrow transplant identified as neutropenic rose to 9.88 per 1000 days at risk during construction activity (July 1989 to August 1992), compared to 3.18 per 1000 days at risk before the construction started (January to June 1989). Installation of wall-mounted portable HEPA filters and implementation of other infection control measures subsequently decreased the incidence of invasive aspergillosis to 2.91 per 1000 days at risk, even though the construction work continued (August 1992 to September 1993). The average concentration of *Aspergillus* in the air during the epidemic period was 6.77 CFU/m$^3$, and no *Aspergillus* was recovered in air samples after the installation of the HEPA filter and the implementation of infection control measures (Loo *et al.* 1996).
- In the fall of 1993, in Israel, a nosocomial outbreak of invasive pulmonary aspergillosis occurred in leukemia patients treated in a regular ward with only natural ventilation during extensive hospital construction and indoor renovation. The infection among acute leukemia patients rose to 50%, and invasive pulmonary aspergillosis developed in 43% of acute leukemia patients during the next 18 months despite the administration of chemoprophylaxis. After that period, a new hematology ward was opened with an air filtration system with HEPA filters, and none of the acute leukemia or bone marrow transplantation patients who were hospitalized exclusively in the hematology ward developed invasive pulmonary aspergillosis, while 29% of acute leukemia patients who were housed in a regular ward, because of shortage of space in the new facility, still contracted invasive pulmonary aspergillosis. The average *Aspergillus* concentration was 0.18 spores/m$^3$ in the new HEPA-ventilated hematological ward, while the average concentration in the regular ward during construction was 15 spores/m$^3$ (Oren *et al.* 2001).

Community-acquired (i.e. out of hospital) opportunistic invasive aspergillosis is not as well documented, but some cases have been reported (Benoit *et al.* 2000; Chen *et al.* 2001). Immunosuppressed patients remain vulnerable to *Aspergillus* infections if exposed in the outpatient setting or at home after being released from hospital (VandenBergh *et al.* 1999).

### 2.2.3 *Allergic bronchopulmonary mycoses* and *allergic fungal sinusitis*

Fungi can colonize the lungs or nasal cavity of patients with underlying respiratory disease such as asthma or chronic rhinosinusitis. This condition is referred to as *allergic bronchopulmonary mycosis* when occurring in the lungs, and as *allergic fungal sinusitis* when taking place in the nasal cavity. Since *Aspergillus* species (especially *Aspergillus fumigatus*) are the most common etiologic agents causing allergic bronchopulmonary mycosis, this condition is commonly referred as allergic bronchopulmonary aspergillosis, or ABPA. Both conditions are characterized by eosinophilia and by the presence of non-invasive fungal hyphae in sputum or in nasal mucus (Hunninghake and Richerson 1994; Ponikau *et al.* 1999). Case reports have suggested a link between fungal counts in the air and the development of acute bronchopulmonary mycoses (Beaumont *et al.* 1984; Kramer *et al.* 1989; Ogawa *et al.* 1997).

## 2.3 Animal Studies

Several experimental studies with animal models exposed to fungal cells, antigens or constituents have found effects similar to those observed in humans in epidemiological studies, such as eosinophilia and increased serum IgE.

- Twenty-four adult albino guinea pigs inhaled daily 8 mg of a *Penicillium chrysogenum* extract nebulized in phosphate-buffered saline (PBS), and two of them were sacrificed each week (up to 12 weeks). Twelve other guinea pigs were handled the same way, but received only nebulized PBS, and one of them was sacrificed each week. No histopathological lesion was found in control animals throughout the experiment, but interstitial infiltrates appeared in the alveoles of *Penicillium*-treated animals after four weeks, and granulomas appeared after 10 weeks. Also, specific IgM and IgG antibodies to *P. notatum* were detectable in *Penicillium*-treated animals after seven weeks (Alonso *et al.* 1998).
- A similar experiment carried out using a *Rhizopus nigrans* extract yielded similar results (i.e. IgG antibodies to *R. nigrans* in serum after seven weeks, and interstitial infiltrates four weeks and granulomas after 10 weeks in the alveoles of *Rhizopus*-treated animals) (Alonso *et al.* 1997).
- Male and female guinea pigs were exposed to aerosols containing either 30 $\mu$g/m$^3$ of (1->3)-B-D-glucan (a

fungal cell component), 75 $\mu g/m^3$ of *Escherichia coli* lipopolysaccharide (a bacterial endotoxin), both, or the vehicle only (controls). Animals were exposed four hours per day, five days per week for five weeks and then sacrificed. Cell counts were determined in Bronchoalveolar lavage (BAL) fluid and in lung interstitium. Macrophages were increased by endotoxin (BAL fluid $p<0.001$, interstitium $p<0.05$) and by glucan+endotoxin (BAL fluid $p<0.001$, interstitium $p<0.05$), but not by glucan alone. Lymphocytes were increased in BAL fluid by endotoxin ($p<0.05$) and glucan+endotoxin ($p<0.01$), but the highest response was observed with glucan alone ($p<0.001$ in BAL fluid and interstitium). Neutrophils were increased in BAL fluid by endotoxin ($p<0.001$) and glucan+endotoxin ($p<0.001$), but not by glucan. Eosinophils were strongly increased by glucan in both BAL fluids and interstitium ($p<0.001$), slightly increased by glucan+endotoxin in BAL fluid only ($p<0.05$) and not affected by endotoxin (Fogelmark *et al.* 2001).

- C57BL/6 mice aged six to eight weeks were sensitized to *Aspergillus* antigens (100 $\mu g$ in 50 $\mu l$ saline) three successive days a week for three weeks, and sacrificed after one, two or three weeks. Control mice were handled the same way, but were administered 50 $\mu l$ saline instead of the antigen solution. Total cells counts were strongly increased in sensitized mice compared to controls at weeks one, two and three ($p<0.02$ at each week). The proportion of macrophages in BAL cells remained constant at 97% over weeks in control mice, while in *Aspergillus*-treated mice the proportion of macrophages decreased (34% at week one) and the proportion of lymphocytes, neutrophils and eosinophils increased. The neutrophil counts in BAL fluid reached $(62.2\pm14.4) \times 10^4$ cells at week two and $(78.2\pm29.1) \times 10^4$ cells at week three in *Aspergillus*-treated mice, compared to $(0.1\pm0.1) \times 10^4$ cells in control mice throughout the study ($p<0.05$) (Wang *et al.* 1994).
- Groups of six to nine female C57B1/6 mice were inoculated intranasally with 50 $\mu l$ saline containing either no fungal conidia (control group), $10^4$ non-viable (methanol-treated) conidia or $10^4$ untreated conidia (of which 25±5% were viable) of *Penicillium chrysogenum* once a week for six weeks, and were sacrificed 24 hours after the last inoculation. *P. chrysogenum* conidia were isolated from a building affected by building-related illness. Mean total IgE levels in serum were 804 ng/ml (SD 301 ng/ml) in controls, 833 ng/ml (SD 339 ng/ml) in animals treated with non-viable conidia, and 2627 ng/ml (SD 1778 ng/ml) in animals treated with viable conidia. Mean percentage of eosinophils over total peripheral white blood cells were 5.3% (SD 0.8%) in controls, 7.3% (SD 1.7%) in animals treated with non-viable cells, and 10.9% (SD 0.9%) in animals treated with viable conidia. Finally, mean eosinophil counts in BAL were 0.22 (SD 0.44) per 1000 BAL cells in controls, 0.5 (SD 0.55) in animals treated with non-viable conidia, and 14.17 (SD 5.91) in animals treated with untreated conidia. Total serum IgE, peripheral eosinophil count and BAL eosinophil counts were significantly higher in the group treated with viable conidia than in controls ($p<0.05$) (Cooley *et al.* 2000).

Also, some studies found severe hemorrhagic responses induced by Stachybotrys chartarum spores.

- Four-day-old Sprague-Dawley rat pups were instillated intratracheally with 1.0 to $8.0\times10^5$ intact spores (suspended in saline) per gram of body weight, similar suspensions of spores treated with ethanol to remove trichothecene toxins, or with saline only. Animals were sacrificed on their 7th or 12th day of life. Cell counts of macrophages, lymphocytes and neutrophils were increased two-fold, five-fold, and seven-fold, respectively, in the bronchoalveolar lavage fluid of animals treated with $1.1\times10^5$ intact spores/g compared to those treated with saline only or with ethanol-treated spores ($p<0.001$ for each cell type). There was no difference between the two latter groups. Hemoglobin concentration in BAL fluids in animals treated with intact spores, ethanol-treated spores and saline were 2.46±0.33 mg/ml, 1.26±0.16 mg/ml and 1.22 mg/ml, respectively; the difference between groups was significant ($p=0.004$). The *S. chartarum* strain used in this study had been isolated in the water-damaged house of an infant that was part of the Cleveland outbreak (Yike *et al.* 2002). These findings indicate that mycotoxins (or another constituent removed by the ethanol treatment) may be responsible for inflammatory and hemorrhagic response of the infant lung to *S. chartarum*.

Other studies with rodents exposed to *Stachybotrys chartarum* showed effects on lung physiology that may be mediated by different mechanisms.

- Carworth Farms White mice were intranasally instilled with 50 ml saline containing either $10^5$ *Cladosporium cladosporioides* conidia per ml, $10^5$ *Stachybotrys chartarum* conidia per ml, or $10^{-7}$ M of isosatratoxin F; another group was untreated (control group). For each treatment, groups of two to four mice were sacrificed 0, 12, 24, 48 and 72 hours post-exposure. None of the mice, regardless of treatment, showed any apparent clinical sign of respiratory distress or sickness. The phospholipid composition

of lung surfactant was significantly modified from 12 hours to the end of the experiment following exposure to *S. chartarum* conidia and isosatratoxin, while following *C. cladosorioides* exposure small changes were observed at 48 hours post-exposure only (Mason *et al.* 1998).

- Groups of five Carworth Farms White mice were intranasally instilled with 50 ml saline containing either $1.4\times10^6$ *Cladosporium cladosporioides* conidia per ml, $1.4\times10^6$ *Stachybotrys chartarum* conidia per ml, or 10 μg/ml of isosatratoxin F, or with saline only. Animals were sacrificed after 24 hours. In vitro conversion of a biologically active form of alveolar surfactant to a biologically inactive form was significantly higher in surfactant of *S. chartarum*-treated mice than in those of all other groups, including controls; other treatment groups were not different from controls with respect to that end-point (Mason *et al.* 2001).
- *Stachybotrys chartarum* spores suspended in saline were instilled into mouse trachea, and mice were sacrificed 24 hours later. Exposure to *S. chartarum* induced an overall reduction of phospholipid content in alveolar surfactant. The relative distribution of phospholipids across surfactant fraction and the nature of surfactant phospholipids were also altered (McCrae *et al.* 2001).
- Groups of five to six mice were inoculated intratracheally with 50 ml saline containing either $1.4\times10^6$ *Cladosporium cladosporioides* conidia per ml, $1.4\times10^6$ *Stachybotrys chartarum* conidia per ml, or 0.02 μg/ml of isosatratoxin F, or with saline only. No difference was observed between alveolar type II cells of control, saline-treated or *C. cladosporioides*-treated animals, while alveolar type II cells from mice treated with either *S. chartarum* spores or isosatratoxin F showed remarkable ultrastuctural changes compared to controls (Rand *et al.* 2002).

## 2.4 Discussion

### 2.4.1 Summary of findings

The major findings on the health effects of mold can be summarized as follows.

- Exposure to indoor mold is associated with an increased prevalence of asthma-related symptoms, such as chronic wheezing, irritative and non-specific symptoms.
- Studies on mold exposure and the development of asthma yielded more conflicting results.
- In laboratory animal studies, instillation of fungal antigens (*Penicillium* and *Aspergillus*) and fungal cell components (1->3-D-glucan) resulted in infiltration of lung tissues by lymphocytes, neutrophils and eosinophils in rodents. Also in laboratory animals, instillation of *Stachbotrys* spores at non-lethal levels resulted in severe biochemical and ultrastructural changes.
- Data published to date suggest that the association between *Stachybotrys chartarum* and acute pulmonary hemorrhage in infants cannot be excluded.
- There is evidence from outbreak investigations and case reports that increased concentrations of airborne fungal spores resulting from environmental perturbations or inadequate control measures are associated with a higher risk of invasive mycoses in immunocompromised individuals. No thorough epidemiological studies have assessed what airborne concentration of *Aspergillus* spores is required to cause an infection.

### 2.4.2 Limitations

*2.4.2.1 Exposure assessment*

In most epidemiological studies on indoor mold and health, the exposure assessment was based on participants' self-reports. In the few studies where exposure to mold was assessed by a member of the research team, the exposure classification was based on dichotomous questions such as the presence or absence of dampness and/or mold; there was no quantitative exposure assessment, and therefore no determination of a dose–response relationship. Exceptions are the studies of Garrett *et al.* (1998) and Dales *et al.* (1999). Also, in most cross-sectional and case-control studies, the mold taxa present in homes were not identified. Mold species differ considerably, not only in their potential to cause adverse effects to human health, but also in the mechanisms by which they can affect health (i.e. through releasing volatile compounds, allergens or mycotoxins) and, therefore, in the nature of effects they can cause.

The difficulty of quantifying human exposure to mold is thus a major obstacle in ascertaining the existence of cause-and-effect relationships, as dose–response relationships cannot be assessed. This difficulty has led the Institute of Medicine (2000) to conclude that ". . . standardized methods for assessing exposure to fungal allergens are essential, preferably based on measurement of allergens rather than culturable or countable fungi . . ." in order to obtain a clear understanding of the effects of building-related fungi.

Quantitative measurement, rather than questionnaire-based assessment, of exposure to fungi may be a promising way to improve epidemiological studies. However, the traditional method of exposure measurement (i.e. air sampling and culture of fungal spores) shows several

limitations that make their utility questionable. For example, airborne fungal spores can be sampled only over short periods of time, while air counts of fungal spores vary considerably over longer periods of time. Also, the culture medium used always favours some species over others, and some fungal taxa have the ability to inhibit the growth of other taxa in culture media.

For all the reasons mentioned above, the determination of surrogate markers of fungal growth, such as ergosterol and (1->3)-ß-D-glucan, in house dust appear to be more promising (Dillon et al. 1999). Both ergosterol and (1->3)-ß-D-glucan are cell membrane constituents in fungi (Li and Hsu 1996; Miller and Young 1997). (1->3)-ß-D-glucan has been associated with increased peak expiratory flow (PEF) variability in asthmatic children (Douwes *et al.* 2000). There is, however, a need for further research to develop standardized protocols for the determination of (1->3)-ß-D-glucan in the environment (Dillon *et al.* 1999). Determination of extracellular polysaccharides (EPS) of *Aspergillus* and *Penicillium* in house dust is another approach being developed for the assessment of exposure to mold. EPS is a fungi-specific marker but, unlike glucan, it is not suspected to be causally related to adverse effects on respiratory health (Chew *et al.* 2001).

Molecular approaches have been developed for assessing both qualitative and quantitative fungal exposure in buildings and other environments (Haugland *et al.* 1999). To date, there is little practical experience with this approach. Some research groups have proposed using animal-derived antibodies to provide quantitative and qualitative information on fungal exposure (Wijnands *et al.* 2000a, 2000b). Another approach to measuring fungal exposure, advocated by the US Institute of Medicine Committee on Asthma (Institute of Medicine 2000), is to determine human fungal allergens or antigens. Research is under way on this in Canada.

#### *2.4.2.2 Outcome assessment*

Objective assessment of health outcomes is another weakness of many epidemiological studies on health effects of mold exposure, since most studies rely on subjective assessments by questionnaires, which once again render the drawing of firm conclusions more difficult. Objective measures of health outcomes do exist, but incorporating them into studies greatly increases study costs.

#### *2.4.2.3 Confounding factors*

Damp conditions favourable to mold growth are also favourable to other biological agents known to be allergenic, such as dust mites and gram-negative bacteria. Unlike mold, bacteria are not visible and, therefore, their presence can be assessed only by air or dust sampling. Therefore, the association observed between mold exposure and allergic responses could be explained in part by confounding bacteria or dust mites being associated with both the exposure to mold and the outcomes considered. This may explain the findings, in some studies (Williamson *et al.* 1997; Norbäck *et al.* 1999), of stronger associations between dampness and asthma than between visible mold and asthma. However, in a cross-sectional study where bacterial endotoxins and dust mites were actually measured, controlling for these other allergen levels did not affect the association between indoor mold and respiratory symptoms (Dales and Miller 1999). Moreover, a case-control study revealed a significant association between mold growth and asthma after controlling for visible dampness (Thorn *et al.* 2001). Also, experiments in animal models showed that mold antigens are able to induce allergic responses in the absence of endotoxin or other biological agents (Alonso *et al.* 1997, 1998; Cooley *et al.* 2000).

Chemical exposures may also be confounders in at least one of the studies summarized above. In Smedje and Norbäck's (2001) cohort study, both airborne fungi and formaldehyde were significant risk factors for incident asthma, but could not be included together in multivariate models because of their strong mutual association.

Other potential confounders in respiratory disease epidemiology, such as socio-economic status, smoking and environmental tobacco smoke exposure, have been controlled for in the majority of cross-sectional and case-control studies reviewed, and are therefore unlikely to explain the findings.

#### *2.4.2.4 Bias*

There may be a reporting bias in some studies, as there is an increasing awareness in the population that molds are suspected to cause respiratory health effects. People with mold problems may pay more attention to symptoms experienced by their children or themselves. This is likely to have occurred in the Finnish cross-sectional study that found an association between mold and backaches and stomachaches (Pirhonen *et al.* 1996). As well, people with respiratory health problems may pay more attention to the presence of mold, as physicians investigating asthma or other respiratory diseases commonly ask patients if they have been exposed to mold or dampness, but this bias was eliminated in many studies by having houses inspected by an investigator blind of participants' case or control status. In the Williamson *et al.* (1997) case-control

study, where the possibility of such a bias was reduced by a case or control classification based on hospital records and exposure assessment based on home visits, an odds ratio of 1.7 (but non-significant) was found between severe dampness and asthma.

*2.4.2.5 Study design*

To our knowledge, only one cohort study was published on health effects of indoor, non-occupational exposure to molds (Smedje and Norbäck 2001). At the time of writing, another cohort study is being conducted in Canada, the Prince Edward Island infant health study. The evidence linking mold to health effects arises mostly from cross-sectional and case-control studies. These two designs are generally considered weaker than cohort studies for investigating the etiology of disease, since it is difficult to ascertain that the suspected cause actually preceded the disease under study. However, though asthma and allergy are chronic conditions, asthma symptoms can improve when exposure to allergens and/or irritants that induce bronchoconstriction is removed. A cross-sectional or case-control study finding an association between "mold and/or dampness" and chronic wheezing does not demonstrate that mold has caused the onset of asthma, but it may indicate that either mold or dampness induces respiratory symptoms in asthmatics (assuming, of course, that both exposure and outcome assessments are accurate; see previous sections). On the other hand, cohort studies of home indoor environments and respiratory/allergic diseases (preferably with objective assessment of exposure and outcome, such as home inspection and physical assessments) are needed to ascertain the existence of a causal link between mold and respiratory diseases.

### 2.4.3 Conclusion

As seen in section 2.4.1, several studies have found significant associations between exposure to mold and/or dampness, and irritative and non-specific respiratory symptoms, as well as the exacerbation and development of respiratory diseases such as asthma. Due to limitations in the assessment of both exposure and outcomes, and since in almost all studies to date an independent effect of mold could not be isolated from that of other contaminants associated with dampness, epidemiologic data alone are insufficient to conclude that indoor mold causes respiratory disease. However, such a causal link is highly plausible in view of the fact that exposure to fungi in occupational environments causes allergic and toxic disease and that adverse effects of fungi have also been seen in inhalation studies using animal models.

In the hospital setting, airborne exposure to certain fungi is associated with an increased risk of fungal infection in immunocompromised individuals.

Although further investigation of health effects of indoor fungi by means of improved exposure and health outcome assessment methods are needed to resolve uncertainties, current knowledge supports the need to prevent damp conditions and mold growth and to remediate any fungal contamination in buildings.

# 3. Investigation of Fungal Contamination of the Non-Industrial Workplace

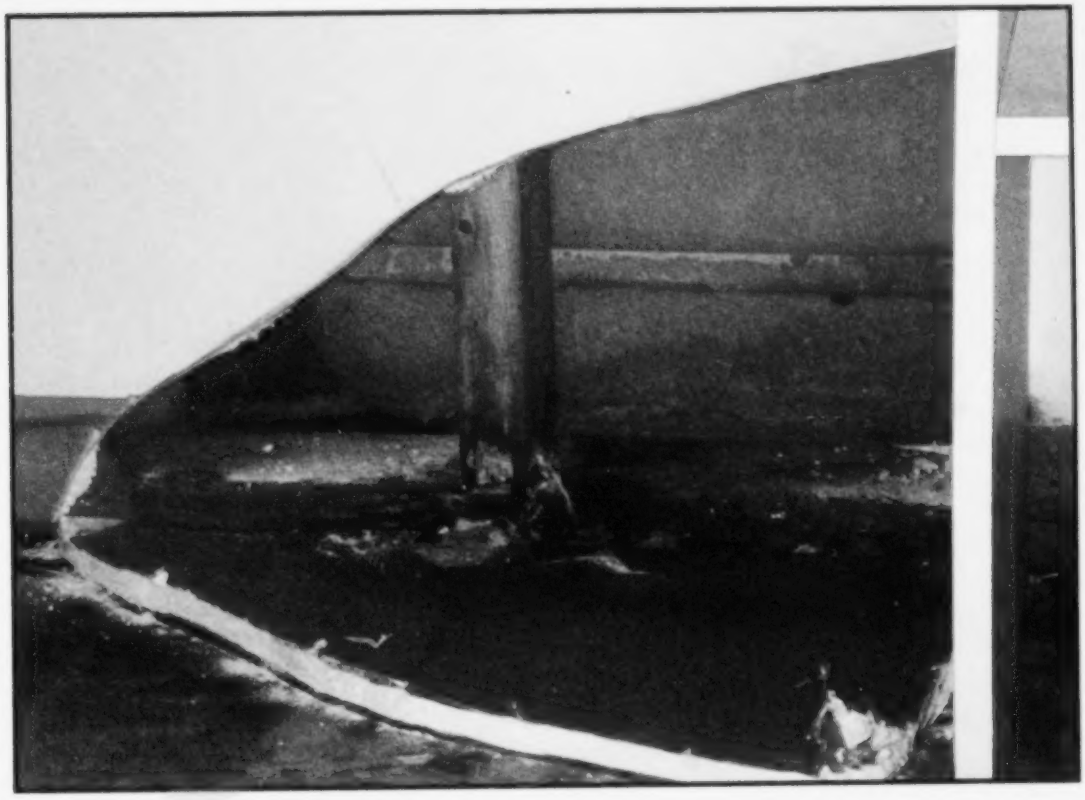

Photo: Architectural Diagnostics Ltd.

## 3.1 Background

A safe workplace is mandated by law in Canada under various legislative frameworks. These include Section 12 of the *Hazardous Products Act*[1], the *Canada Labour Code*, the *Transportation of Dangerous Goods Act*, provincial occupational health and safety acts, and related regulations. It is essential to have in place an operating procedure that will protect the health and safety of occupants[2], as well as the workers performing their duties in the investigation of possible fungal contamination in public buildings[3] (CEOH 1995a). Analyses of the legislative framework for indoor air quality (IAG) in Canada illustrated its variable nature and described the case law that might apply (Beaudry 1999; Morton and Kassirer 2000).

Recent reviews indicated that there is no specific regulatory mention of most contaminants present in residential or office indoor air (CEOH 1989, 1995). As is the case with other indoor air contaminants, the legal framework for mold is mainly based on regulations that suggest or require the adherence to the advice of cognizant authorities, including CEOH, the American Society of Heating Air-Conditioning & Refrigerating Engineers Standard 62, and the ACGIH Threshold Limit Values (TLVs), as well as determinations or policies of provincial and territorial labour and health departments. In addition, health and safety requirements in legislation impose some obligations to industrial hygienists, professional engineers, physicians and other health professionals to act in accordance with the best interests of occupants. This should be done in accordance with the policies of the designated Medical Officer of Health or Public Health Directors for the area concerned and/or Health Canada for federal jurisdictions.

It cannot be emphasized enough that the best way to manage mold growth is to prevent it before it occurs. The essential elements of a prevention strategy are the control of moisture, the timely remediation of any water leakage, and adequate maintenance of heating, ventilation and air conditioning (HVAC) systems (Lavoie and Lazure 1994; Flannigan and Morey 1996).

1. Which established the Workplace Hazardous Materials Information System (WHMIS).
2. In section 3, "occupants" means individuals present in public buildings, including workers, students, visitors and the general public.
3. In section 3, "public building" means any building accessible to the public (e.g. office building, school, store).

## 3.2 General Principles

Indoor air quality investigations[4] can begin in several ways. Some building owners or managers conduct regular air quality audits to detect problems before they can potentially affect occupants. At the other end of the spectrum are investigations which occur as a result of acute reactions from individuals entering a building. Appropriate measures should be taken when an investigation is prompted by health complaints. It is important to involve specialists with recognized professional training and experience to investigate potential mold problems in public buildings using methods documented by the ACGIH (1999) and the American Industrial Hygiene Association, or AIHA (Dillon *et al.* 1996).

In HVAC systems, humidifiers, dirty filters and accumulated debris in ducts subject to condensation or leaks can all be sources of building-associated mold. Spores can be blown out of ducts in a periodic fashion. Fungi can be released when occupied spaces adjacent to contaminated wall cavities, elevator shafts or faulty sewer drains are depressurized. Release from these sources can be affected by air infiltration rates and pressure differentials resulting from wind and thermal loading (weather) and unbalanced ventilation or exhaust systems. Distribution of fungi from carpets or surface contamination is affected by activity in the occupied space and the intensity of cleaning.

If required, laboratory tests must be done using appropriate methods and by qualified and experienced professionals. Commercial laboratories should demonstrate successful performance in the AIHA Environmental Microbiology Proficiency Analytical Testing (EMPAT) program and preferably be an Environmental Laboratory accredited by the Standards Council of Canada (SCC),[5] or ISO or Good Laboratory Practice (GLP) certification.[6]

4. In section 3, "investigation" means the process of appropriately trained individuals entering the building to conduct inspection, sampling, documentation and production of reports.
5. The Standards Council of Canada (SCC) offers environmental laboratory accreditation in partnership with the Canadian Association for Environmental Analytical Laboratories (Inc.) (CAEAL). CAEAL is a not-for-profit association of public and private sector laboratories.
6. Good Laboratory Practice (GLP) refers to compliance with a series of guidelines, developed by the Organisation for Economic Co-Operation and Development (OCED), regarding laboratory facilities, standard operating procedures (SOPs), quality assurance and reporting. GLP certification is granted by a number of agencies around the world, including the US Food and Drug Administration.

There are also university and governmental laboratories with highly qualified specialists in mold identification that can provide reliable data; however, they must be required to use recognized methods. Canada Mortgage and Housing Corporation (CMHC) attempts to maintain a list of laboratories that have provided services for the Government of Canada. Provincial officials in Health or Labour Departments may also be able to provide recommendations. All reasonable steps must be taken to ensure that no action during the investigation or remediation[7] process results in further contamination of the building or increased risk for occupants or the public. Finally, the provision of reliable and timely information to occupants is a critical aspect of any IAQ investigation because of the need for individuals in one of the potential risk groups to be informed in the event of a microbiological problem in their workplace or education facility.

## 3.3 Objectives of a Mold Investigation

As noted above, the intensity and complexity of mold investigations vary according to the size and nature of the building, whether an air quality audit is being conducted or whether the investigation is a response to a health complaint. The benchmark is the CEOH (CEOH 1989, 1995a, 1995b) advice to minimize exposure to fungi, that there are population health effects of mold and dampness and that there are risk groups. The goals are to define and manage the microbial problem(s) and return the building to a satisfactory level of performance. Air quality investigations for audit purposes are not considered here.

The following discussion refers to an incident in which a health complaint has been made and initial evidence shows that mold might be one of the potential issues.

The goals of such investigations are to:

- establish the cause, nature and extent of fungal contamination;
- assess the risk of adverse effects on the health of occupants;
- manage the microbial problem(s); and
- return the building to a satisfactory level of performance.

*Factors to consider when addressing a potential mold problem*

1. It is important to focus on the problem as quickly as possible to allow the investigating team to provide clear answers to the occupants, managers and health providers about the state of the building. For reasons discussed in section 3.4, mold problems very often result from chronic moisture problems. If exposure has reached the point where health complaints have been made, it has typically taken years for this to occur. Since there is some mold on materials in all buildings, determinations must be made regarding the nature and extent of the contamination and who is exposed.
2. The first step in a mold investigation is an informed inspection during or after which air samples may be taken (see 3.4.1). Investigators must decide whether there is a possible hidden contamination, including in the HVAC system or wall cavities (Miller 1993). In such cases, air samples are especially useful. Air samples collected using methods that require the culturing of viable spores are often sensitive even to subtle problems, and provide results after 7 to 10 days. In contrast, non-viable (sticky surface) samples are generally less sensitive, but provide data within 24 to 48 hours. All samples should be taken according to the methods described in the AIHA Field Guide (Dillon *et al.* 1996).
3. After air samples are taken, floors and other surfaces where dust may accumulate should be cleaned with a high efficiency particle arresting (HEPA) vacuum. With few exceptions, most exposure to mold spores arises from people moving around in the occupied space which stirs up settled dusts. Cleaning will, in most cases, immediately reduce exposure while the investigation process continues. If there are people in the building, the decision of whether or not to take air samples should be made quickly so cleaning can proceed.
4. As the nature and extent of the contamination become known, this information needs to be given to occupants. Among other things, this permits those with special sensitivities to unusual airborne mold exposures to consult with a medical professional about whether they should remain in the building, or not.
5. An accurate survey of the extent of the contamination and moisture or damage is required to document and remediate the affected area. There are three purposes for this important step: (a) the complexity of the removal process depends on the affected area (ACGIH 1999; New York City Department of Health 2000); (b) there is evidence that the potential for health effects among occupants depends on the area

7. "*Remediation*" includes both the thorough cleaning of any mold growing in the building and correcting the building defect that led to mold growth (excessive humidity, water leaking, or water infiltration from the outside).

of mold contamination; and (c) the quality assurance process for the remediation depends in part on the thoroughness of the investigation (AIHA 2001). During the investigation stage, dust control (e.g. misting and using a HEPA vacuum) is required when making small investigative openings in walls ($< 0.1$ m$^2$) adjacent to occupied space. Larger inspection holes, especially where mold contamination has been determined to be extensive, requires containment or other protective measures if the space is to be re-occupied before doing repairs. The survey should also assess the degree of connection of the discovered mold to the living/working space. The documentation step can be eliminated if the building has been unoccupied.

6. In documenting the nature and extent of the mold observed, there are several reasons for determining the fungal species present. The first reason is to verify that the damage seen is really fungal in nature. Sometimes dirt or other deposits on surfaces in buildings or HVAC diffusers are not building-associated mold. It can also be useful to know what environmental conditions have led to mold growth. The species of mold present can provide valuable clues. Molds that grow under damp conditions are different from those that grow under very wet conditions. This might help in identifying sources of water that might not be obvious. For example, the finding of a wet-loving building-associated mold near a window frame might suggest a significant leak around the window or a condensation problem in the wall cavity. The finding of a damp-loving mold behind a chest of drawers on an outside wall might suggest inadequate ventilation or insulation. In addition, the occupants' medical professional may find it useful to have a list of the dominant fungi present.

7. Fungal damage should be quickly remediated using the protocols outlined in the New York City Department of Health Guidelines (2000) and ACGIH guidelines (1999; Chapter 15) (ACGIH 1999; New York City Department of Health 2000) and quality assurance process used (AIHA 2001). These are similar to those outlined in the CEOH Guide (1995a). Note that the primary method to assure that mold remediation has been done properly is confirmation that the causal water or moisture sources have been identified and eliminated. Repairing mold damage in an exterior wall where it has been determined that there is a good air barrier can typically be done without additional protective measures. The air barrier should normally be sufficient to prevent the ingress of mold spores indoors. It is important to note, however, that construction, demolition and repairs all result in the release of construction dusts and other debris. It is generally not appropriate to permit the space to be occupied when construction is actively under way. Measures to protect contents from settled dusts during repairs, plus HEPA cleaning after repairs, are additional steps potentially required for re-occupancy, depending on the nature and extent of the damage and repairs. Containment of dust and spores using negative pressure and isolation of the remediation area is a prudent practice. There is no public health reason to contain the outside of a building in the situation where remediation is being done from the exterior. A possible exception is when the exterior walls are in a semi-enclosed space (e.g. a stairwell).

8. The risk of health consequences from mold exposures arising from mold-damaged building materials varies with the degree of isolation from the occupied space. Considering construction methods and climates in Canada, exposure risk from greatest to least would be growth: (a) on surfaces exposed to occupied space; (b) on interior walls or floor cavities (especially if there are ducts); (c) in exterior walls with poor air barriers; (d) in exterior walls outboard of a good air barrier; and (e) in attic space or roof space above an air barrier. If the mold damage is in the ventilation system, immediate steps are required to stop the spread of contamination. If the contamination is on the surface of walls, ceilings or floors exposed to the occupied space, immediate steps are required to contain the mold-damaged areas. Options to consider are the use of polyethylene barriers either with or without depressurization. If the contamination is mainly in demising wall cavities, access to highly damaged areas of the building should be restricted until remediation is complete. In the remaining areas, professional judgment will have to be made on the potential to introduce contamination into the occupied spaces until repairs are complete. Additional steps to consider include regular HEPA cleaning with some air monitoring to ensure the effectiveness of the cleaning. A team to manage the remediation and repair process needs to be created. The remediation and repair work should be closely monitored to ensure its effectiveness, and quality assurance and compliance testing should be incorporated.

9. Typically, all porous materials on which there has been fungal growth must be safely and effectively removed, followed by a thorough particulate cleaning by crews appropriately trained in dust control. Surfaces that collect large amounts of settled dusts and spores, including carpets and ceiling tiles, may also need to be removed. These are difficult to clean and there are no accepted methods of verifying that they

have been adequately cleaned. Factors to consider in making the decision to remove remaining porous surfaces in mold-damaged areas include the nature, extent and duration of the mold problem in the building. As remediation proceeds, exposed areas must be checked for any remaining mold damage revealed during demolition. Semi-porous materials on which mold growth has occurred can be cleaned if they are structurally sound, and must be replaced if they are not. Dusts on non-porous surfaces can usually be cleaned using methods appropriate for the given material.

If the contamination was extensive (i.e. on the upper end of the remediation categories defined by ACGIH [1999] or New York City Department of Health [2000]), settled dust samples should be collected after remediation. The purpose of this step is to provide documentation that the affected areas have been thoroughly cleaned. The dry weight of settled dust collected/$m^2$ should be measured according to the guidance from the AIHA (2001). When the mold-damaged materials have been removed and the affected area thoroughly cleaned with a HEPA vacuum,[8] the building can be treated like a normal construction site for the build-back.

After the repairs have been completed, air samples taken one or two weeks after the ventilation system has been running normally can be useful as a last measure of the success of the steps taken.

## 3.4 Methodological Considerations

Microbiological sampling during a building investigation for mold-related problems is complicated. Several cognizant authorities have published guidance on this topic. For example, the AIHA published a Field Guide of consensus methods for microbiological sampling (Dillon *et al.* 1996). The ACGIH has published a comprehensive manual on microbiological problems of buildings, including chapters on investigation and remediation that recognize the AIHA manual as a source for sampling methods (ACGIH 1999). Details on some of the procedures can also be found in Flannigan, Samson and Miller (Flannigan et al. 2001). The US Environmental Protection Agency (EPA) has also published mold remediation guidance for public buildings (USEPA 2001) and for homes (USEPA 2002).

The goals of any investigation are to establish the cause, nature and extent of fungal contamination and to assess the risk of adverse effects on the health of occupants.

### 3.4.1 Informed inspection

The first step in investigating a building for microbial contaminants is an informed inspection. This should be performed by someone with engineering or architectural knowledge of moisture problems in buildings, considering the type of building under investigation. The investigation of large public buildings requires a different skill set than a house. Mold contamination can arise from condensation, floods and various types of leaks. Inspection of mold problems requires a thorough knowledge of the design of the building envelope and of the types of failures that result in condensation and water leaks. The physical investigation of molds in both public and domestic buildings requires considerable expertise in the design, construction and operation of these structures. Informed inspection checklists suitable for residential housing have been developed by CMHC (1993) and by Public Works and Government Services Canada (Davidge *et al.* 1992) and the USEPA (USEPA and USDHHS 1991) for public buildings.

Air sampling is not appropriate unless a thorough building inspection is done either on a concurrent basis or before sampling. Sampling is done to identify contamination that is not visible without destructive testing and to document air contamination. Similarly, after sample results are obtained, the data must be compared with the information obtained during the physical inspections. "Are the results plausible?" is a question that must always be asked and answered to properly assess the risk of false negative and false positive results for mold contamination. Additionally, documentation of the sources and nature of the contamination allows a failure analysis to be done on the building (or HVAC system). This will assist in developing cost-effective investigation strategies and ultimately any remedial action necessary.

### 3.4.2 Culturable air samples

Air samplers collect fungal propagules either on agar media or in aqueous suspensions. Such samples provide information on culturable or "viable" propagules in air. It is important to consider that existing air-sampling techniques underestimate the true airborne concentrations of fungal spores for several reasons. The number of fungal propagules determined by culture are substantially less (by 1% to 50%) than those determined by direct methods; however, this varies between species. Different species of fungi have different growth requirements so the use of any

8. Residual dust should be reduced to as low as can reasonably be achieved. This has been defined as less than 100 mg dry weight/$m^2$ on smooth surfaces in a number of cognizant authority documents (ACGIH 1999; AIHA 2001).

medium produces different recoveries. The spores of fungi decline in viability with time; the spores of some species remain viable for years and for other species for months. Some species grow very fast or are aggressive in culture and produce antifungal agents that can affect the growth of other species present in culture. The variability of spore clouds in the air in a building with active mold growth is much larger than the precision of available sampling methods. Air sampling is useful for investigating large buildings for mold contamination and must be considered if the investigation was prompted by health complaints.

It is seldom possible to take enough samples to conduct rigorous statistical analyses, but statistical principles need to be considered when determining the number of samples to be taken (ACGIH 1999). Careful consideration must be given as to how and where each sample is to be taken.

Air samples should be taken during normal activity in the building, while the ventilation system is operational. Factors to consider include taking samples in a given space and allowing one or two hours between duplicates (e.g. go around each floor of the building in one direction, go up each level and then down, morning and afternoon, etc.). This technique takes into consideration the variability of airborne spore concentrations over time and with different activities, as well as varying thermal and wind loads. Air samples should not be taken when it is raining. Rain has a transient effect on the microbial populations in outdoor air that can result in a reduction of the sensitivity of the indoor–outdoor comparison. The number of outdoor air samples should in principle be equal to the number taken indoors. Since this is seldom practical, there needs to be at least between three and six samples taken outdoors during the period(s) when the indoor sampling is under way. These need to be taken above grade to avoid collecting windblown soil particles containing fungi which can affect the comparison of the indoor–outdoor diversity. It is recommended that outdoor air samples be collected as close to the air intake as possible or facing into the wind on the building roof. Other considerations can be found in the AIHA Field Guide (Dillon *et al.* 1996) and the ACGIH bioaerosols manual (ACGIH 1999).

The basis of the current methods for interpreting the results of air sampling is a comparison of the diversity of the fungi inside with outdoor air samples, taking into account indicator species and species with poor recoveries on agar media such as *Stachybotrys chartarum* (CEOH 1995a; Dillon *et al.* 1996; ACGIH 1999). There is a shifting array of fungal species in outdoor air as the season progresses. Average numbers of total propagules in July range from 20,000 per $m^3$, to peak levels of twice that value. In the absence of snow cover, total *Aspergillus/Penicillium* comprise <1% of the total fungal spores present in outdoor air. When there is snow cover, the total number of fungal spores decreases, and the proportion of *Aspergillus/Penicillium* therefore increases to 10% to 20%.

The advantage of properly collected and analyzed viable air samples is that the data can be used to detect signs of the early stages of a mold problem, as well as growths in wall cavities or ventilation ducts (where dilution by outside air limits the sensitivity of the analysis).

### 3.4.3 Sticky surface samplers

Sticky surface samplers such as Zefon Air-O-Cell™, Allergenco™ and Burkhard™ are increasingly used in IAG investigations. There is little published information on their comparative quantitative and qualitative performance (Dillon *et al.* 1996). However, some studies have provided information on the cut points of these samplers (Aizenberg *et al.* 2000). A limitation of these methods is the skill of the microscopist in counting fungal propagules in a field containing debris of various kinds.

Advantages of data from properly collected and analyzed sticky surface samples include the fact that the results are available within a day and in situations when there is a high percentage of non-viable spores in the air, the data are more reliable.

### 3.4.4 Documentation of visually moldy area

Within the informed inspection component of the overall investigation, detailed notes of the amounts of mold visible should be noted on the appropriate perspective of the building plans. The moldy areas should be drawn on the plan with sufficient accuracy to permit an estimation of the number of square metres of mold.

Bulk samples might be collected from the visibly moldy building materials to delimit the affected areas by examining the materials for fungal growth. A small amount of material can be scraped off the surface and examined under the microscope and/or plated on agar media. Usually, the colour of visibly moldy material comes from conidia, ascocarps, pycnidia and, in the case of melanized fungi, the mycelia. Conidia that are not visible to the naked eye, but present on building materials may still have the potential to affect the air quality of the occupied space.

Where there is probable cause to believe that there is appreciable mold behind wall cavities, physical inspections

should be performed by opening up the hidden area. Factors to consider include whether there is insulation in the walls and what kind of water damage has occurred. For example, if there has been a pipe burst, flood, fire storm or evident problems with the cladding or windows, all affected areas can be reasonably suspected of having been affected and need to be examined for mold damage. The informed inspection and/or air samples can be useful in determining whether destructive testing is required. Methods range from sawing the bottom 0.3 metre off one side of interior walls to using a keyhole saw and a boroscope (AIHA 2001). Such destructive testing should be done with source control HEPA vacuums (e.g. near the saw) or under simple containment, using all appropriate respiratory protection required (ACGIH 1999).

### 3.4.5 Mycological analysis of bulk samples

Bulk samples refer to physical, destructive samples of building materials. Dilution plating methods are selective and do not provide direct information on the fungi growing on the damaged material versus dormant organisms that might have settled out from the air. Dilution plating involves taking an amount of a powered material (e.g. ground wallboard, settled dust) and suspending it in an appropriate diluent. This is then further diluted in 10-fold steps and aliquots are plated on agar media at least in triplicate, followed by spreading the liquid evenly over the surface, incubating and counting the colonies that emerge. Representative colonies are then transferred to agar media appropriate to identify the species present. The strength of this method is that a picture of the diversity of species present can be obtained.

In the absence of regular HEPA vacuum cleaning, mycological analysis of settled dust samples has value in identifying a problem that might be seasonal or due to factors not present at the time of the investigation (ACGIH 1999). For example, condensation around perimeter induction units, which leads to wet carpets, occurs only in the summer. Investigation of occupant complaints may occur when the carpet is not wet. As is the case for air samples, interpretation of dust samples is appropriately made by analysis of the fungal diversity, but is less straightforward (Dillon *et al.* 1996; ACGIH 1999).

Small pieces of building materials collected (ca. 0.5 g) can be plated on different agar media. These are incubated and the colonies that grow out are counted and transferred for identification. The advantage of this method is that the colonies that first emerge from moldy building material are likely to be the most reflective of those active in the damaged material.

### 3.4.6 Microscopic techniques

Samples of moldy building materials that are plated by either method should also always be mounted in lactophenol cotton blue or other appropriate stain and examined by microscopy to determine the presence of organisms that might not be viable. This allows a comparison to be made between viable and non-viable cultures. This will provide information on the dead fungi present on damaged material to be obtained, thus helping prevent false negative results. (Dead fungal spores still contain allergens and toxins.) If the majority of the fungi are found to be dead on a moldy item, the water event probably occurred months to years ago.

There are two basic techniques to examine moldy surfaces by microscopy: tape samples and mounting scrapings of the mold-damaged area collected in small plastic bags or vials. Tape samples are made by pressing the affected surface with good quality cellophane tape. If scrapings are available, they can be mounted on slides and examined; they can also be cultured whereas the tape samples cannot. As with all microscopic methods, large dark spores are easier to see and, depending on the skill of the microscopist, small hyaline spores are often overlooked. The taxonomic information obtained is limited.

## 3.5 Conclusion

The guidance offered above on remediation and inspection emphasizes the need to use investigators who are qualified and experienced in this aspect of engineering and industrial hygiene. Because there is considerable variation in construction methods used and in climates across Canada, such investigations cannot be standardized in detail.

The guidance on mold sampling has emphasized that sampling is often a necessary part of investigation for public buildings and less useful for house dwellings.[9] Sampling should be done by qualified and experienced investigators using laboratories with demonstrated proficiency.

As noted in most current documents concerning mold in buildings, prevention is key. Prompt attention to condensation and water leaks in the building fabric, and wet building materials (resulting from plumbing or other causes, such as flood or storm damage) will eliminate the growth of mold and prevent the increase of other contaminants, such as house dust mites in the built environment. Such preventive actions are relatively inexpensive compared to the costs associated with remediation of mold problems in buildings. The value of prevention appears even more obvious when one takes into account health problems that may be avoided.

9. CMHC has posted a discussion of the merits of mold sampling in single family dwellings on its Web site: http://www.cmhc-schl.gc.ca

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